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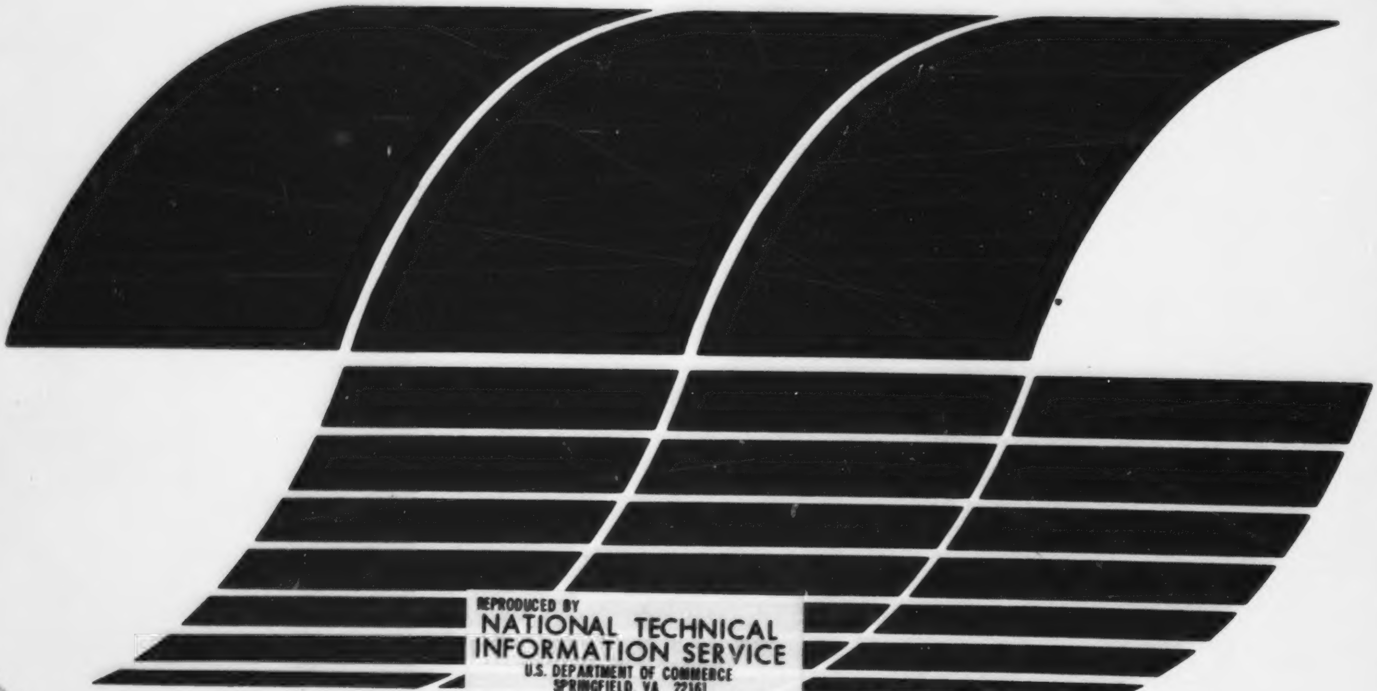

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16. ABSTRACT

This reports describes the results of field and laboratory investigations conducted between October 1978 and October 1980. The field studies yielded data on the prevalence and geographical distribution of diseased bottom-dwelling flatfish, specially English sole and starry flounder, in the Duwamish Waterway, Seattle, and other selected estuaries in Puget Sound. Levels of toxic metals, chlorinated hydrocarbons, and polynuclear aromatic hydrocarbons (AHs) in fish and bottom sediments from these estuaries were also determined. The laboratory studies were conducted in order to evaluate cause-and-effect relationships between sediment-associated chemicals and the abnormalities observed in English sole.

A variety of lesions were observed in the liver, kidneys, gills, gastrointestinal tract, and in the skin and fins of both species from the urban estuaries. The types of lesions found in other organ systems were either found at very low prevalence or the geographical distribution of animals with these lesions was such that clear-cut associations with urban areas were tenuous. Concentrations of polychlorinated biphenyls (PCBs) in liver tissues of both English sole and starry flounder reflected the concentrations of these compounds in sediments from which the animals were captured. This study constitutes an important step in further defining the pathologic anatomy and geographical distribution of several urban-associated abnormalities in two flatfish species which are widely distributed in Puget Sound.

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PATHOLOGY OF TWO SPECIES OF FLATFISH FROM URBAN ESTUARIES IN PUGET SOUND

by

Bruce B. McCain, Mark S. Myers, Usha Varanasi,
Donald W. Brown, Linda D. Rhodes, William D. Gronlund, Diane G. Elliott
Wayne A. Palsson, Harold O. Hodgins and Donald C. Malins

Environmental Conservation Division
Northwest and Alaska Fisheries Center
National Marine Fisheries Service
National Oceanic and Atmospheric Administration
2725 Montlake Boulevard East
Seattle, Washington 98112

NOAA Project Officer: Douglas A. Wolfe (NOAA/Boulder, CO)

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FOREWORD

Human activities in or near coastal areas create a diverse variety of waste materials and by-products, which may eventually reach the marine environment and have negative impacts on marine resource values. In order to develop an adequate understanding of the potential marine environmental consequences of such pollution, the National Oceanic and Atmospheric Administration has conducted studies on the Fate and Effects of Petroleum Hydrocarbons and Toxic Metals in Selected Marine Organisms and Ecosystems under Interagency Agreement with the Environmental Protection Agency. The overall objectives of this effort have been to study experimentally those specific processes underlying the distribution, transport, and biological effects of petroleum hydrocarbons in coastal marine ecosystems. The results are expected to facilitate the assessment of impacts of contaminant releases, and thereby to improve the basis for developing regulatory measures for suitable protection of the marine environment.

The effects of chemical pollutants on the ecosystem and animal populations in oceans and estuaries are of concern, with respect to loss of marine animals and to possible harmful effects on man as a result of eating contaminated food or by direct contact with water. One means of determining the environmental factors which may lead to carcinogenic or toxic effects on marine animals is to investigate sensitive marine animals for pathological conditions which are associated with polluted areas. The report that follows describes disease prevalences in flatfish from relatively contaminated and uncontaminated estuarine systems in Puget Sound, along with results of experiments designed to test cause/effect relations between sediment-associated chemicals and the observed pathological abnormalities.

Douglas A. Wolfe
Office of Marine Pollution Assessment
NOAA

ABSTRACT

Described in this report are the results of field and laboratory investigations conducted between October 1978 and October 1980. The field studies yielded data on the prevalence and geographical distribution of diseased bottom-dwelling flatfish, specifically English sole (Parophyrys vetulus) and starry flounder (Platichthys stellatus), in the Duwamish Waterway, Seattle, and other selected estuaries in Puget Sound. Levels of toxic metals, chlorinated hydrocarbons, and polynuclear aromatic hydrocarbons (AHs) in fish and bottom sediments from these estuaries were also determined. The laboratory studies were conducted in order to evaluate cause-and-effect relationships between sediment-associated chemicals and the abnormalities observed in English sole.

A variety of lesions were observed in the liver, kidney, gills, gastrointestinal tract, and in the skin and fins of both species from the urban estuaries. In addition to the Duwamish Waterway, these urban estuaries included the Lake Washington Ship Canal, Seattle, and the Snohomish River, Everett. Fish with some of these lesions were also found in McAllister Creek, the reference estuary, near Olympia.

Fourteen principal types of idiopathic liver lesions were observed: eight of the lesions were non-neoplastic, two were classified as potentially preneoplastic and four were clearly neoplastic. English sole and starry flounder with the latter two types of lesions were found only in the Duwamish Waterway and the Lake Washington Ship Canal. The only liver lesions found in flatfish from the Snohomish River and McAllister Creek were non-neoplastic lesions: nonspecific hepatocellular necrosis, fatty change (an abnormal accumulation of lipid in hepatocytes), hemosiderosis (an intracellular accumulation of iron-containing pigments), and intrahepatic blood cysts. The prevalence of all these idiopathic liver lesions was very low in the latter two estuaries.

The prevalences of English sole with either "preneoplastic" or neoplastic lesions, or both, were 20.5% (113 of 551 fish) and 20.4% (10 of 49 fish) for the Duwamish Waterway and Lake Washington Ship Canal, respectively. The prevalence of English sole with liver neoplasms in these two estuaries was 12.9% (71 of 551 fish) and 8.4% (4 of 49 fish), respectively. In the Duwamish Waterway, the prevalence of starry flounder with liver neoplasms was 1.1% (3 of 279 fish), considerably lower than that of English sole. (This is the first report of liver neoplasms in starry flounder.)

The types of lesions found in other organ systems were either found at very low prevalence or the geographical distribution of animals with these lesions was such that clear-cut associations with urban areas were tenuous. The four most common idiopathic lesions of the gills were respiratory epithelial hyperplasia (a thickening of the respiratory epithelium), lymphocytic infiltration (a chronic inflammatory response

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to injury), microaneurysms (small dilations of blood vessels, probably a shock response induced by capture), and mucous cell hyperplasia (an increase in the density of goblet cells in gill lamellae and filaments). The two most prevalent kidney lesions were mesangiolysis and mesangiosclerosis, both being primary lesions of the glomeruli. Pathological conditions of the gastrointestional tract generally were associated with parasitic infestations, and affected animals were usually found in all the estuaries. Lesions of the skin and fins included such grossly visible conditions as parasitic infestation (nematodes and trematodes), lymphocystis (a virus-caused condition), skin tumors (growths which may be caused by a parasitic amoeba), and fin erosion.

Concentrations of polychlorinated biphenyls (PCBs) in liver tissues of both English sole and starry flounder reflected the concentrations of these compounds in sediments from which the animals were captured. In the Duwamish Waterway, the liver tissue levels of PCBs in English sole ranged from 17.0 to 161.0 μg/g (dry weight), compared to 0.5 μg/g (dry weight) in a composite of English sole livers from McAllister Creek. Low concentrations of most AHs were detected in the livers of flatfish from both the urban and reference estuaries. This observation of concentrations of AHs in fish in the urban estuaries was almost surely a reflection of the well-documented ability of flatfish to rapidly metabolize AHs to compounds not detectable by routine analytical procedures. Nevertheless, one AH, naphthalene, was frequently detected in liver tissue of English sole from the Duwamish Waterway, but not in sole from McAllister Creek, the reference estuary.

Three types of laboratory experiments were performed to evaluate if English sole were adversely affected by exposure to contaminated bottom sediments. In one type of experiment, sole were maintained for up to 3 months in aquaria containing either sediment from the Duwamish Waterway or from a minimally contaminated area. The mortality rate of sole exposed to sediment from the Duwamish Waterway was not statistically different from that of sole on reference sediments. Another type of experiment involved intraperitoneal injection of juvenile English sole with extracts of contaminated or reference sediments and observing the fish for possible effects for up to 3 weeks. Extracts of sediment from the Duwamish Waterway were more acutely toxic than were extracts of reference sediment; significantly ($p \leq 0.05$) more fish died when injected with extracts of Duwamish Waterway sediment than did fish receiving reference extracts, carrier (corn oil) only, or no injection. None of the above-mentioned major histopathological conditions observed in English sole from the Duwamish Waterway were observed in experimental fish. The third type of laboratory experiment was intended to define the uptake from sediment and fate of a carcinogenic polynuclear AH, benzo[a]pyrene (BaP), by English sole. English sole were exposed for 24 hours to reference sediment contaminated with radioactively-labeled BaP; concentrations of BaP in the blood, liver, muscle, and gall bladder were measured. The highest levels of BaP and its metabolites were detected in bile and in liver tissue.

This study constitutes an important step in further defining the pathologic anatomy and geographical distribution of several urban-associated abnormalities in two flatfish species which are widely distributed in Puget Sound. Although laboratory experiments were initiated in an attempt to elucidate the causes of these abnormalities, the causes remain unknown. These experiments do provide evidence, however, that sediments from the Duwamish Waterway contain factors which can be toxic to English sole.

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SECTION 1

INTRODUCTION

Pathological conditions have been reported in demersal fishes in the Duwamish Waterway: (1) liver neoplasms in English sole (Parophrys vetulus) (McCain et al. 1977, Pierce et al. 1978); (2) skin papillomas of English sole and starry flounder (Platichthys stellatus) (Wingert et al. 1976, Wellings et al. 1976b); (3) fin erosion of starry flounder and English sole (Wellings et al. 1976a); and (4) non-neoplastic liver abnormalities (including severe hepatocellular lipid vacuolization, necrosis and loss of liver structure) in 90% of both the starry flounder and English sole (Pierce et al. 1978, Wingert et al. 1976). The Duwamish estuary receives agricultural, industrial, and municipal wastes. Its sediments are known to contain carcinogenic aromatic hydrocarbons (AHs), such as benzo[a]pyrene (BaP) and benzanthracene (Malins et al. 1980), heavy metals, and high levels of polychlorinated biphenyls (PCBs) (Pavlou et al. 1973, Pattie 1975, Sherwood and McCain 1976, Malins et al. 1980).

Although at present there is no unequivocal evidence that the above-mentioned pathological conditions are caused by xenobiotic chemicals, microbiological and histological evidence suggests that infectious microorganisms are not the direct cause. Thus, environmental pollutants seem to be the most likely etiological agents.

Field studies were performed over a 2-year period in the Duwamish River estuary (Duwamish Waterway), Lake Washington Ship Canal, Snohomish River estuary (urban-associated areas), and in a reference area, McAllister Creek to (1) describe the types and prevalences of histologically detectable pathologic conditions in English sole and starry flounder captured in these areas, (2) define the ecology of these species in the respective sampling areas, and (3) investigate the potential relationship of these pathologic conditions to levels of xenobiotic chemicals present in the sediments from these areas and/or in tissues from affected fish.

Three types of laboratory studies were also conducted to evaluate if English sole were adversely affected by exposure to contaminated sediments. The long-term effects of such exposure were tested by maintaining fish on sediment from the Duwamish Waterway for up to 3 months. The acutely toxic effects of components of sediment from this waterway were tested by injecting sole with extracts of fractions of sediment. The third type of study was intended to define the relative uptake of BaP from contaminated sediments by English sole.

SECTION 2

CONCLUSIONS

1. English sole and starry flounder with certain types of lesions of the liver, gills, kidney, and skin were found primarily in urban estuaries of Puget Sound. The lesions of the liver included neoplastic, "preneoplastic", and a variety of non-neoplastic lesions (e.g., megalocytic hepatosis, hepatocellular regeneration and hyperplasia, and cholangiofibrosis). These idiopathic liver lesions were found exclusively in fish from the Duwamish Waterway and the Lake Washington Ship Canal, both located in Seattle. Flatfish with a gill lesion, (respiratory epithelial hyperplasia), a kidney lesion, (peritubular fibrosis) and a skin lesion, (epidermal necrosis), were found in these two estuaries as well as in the estuary of Snohomish River in Everett. Most idiopathic lesions in the liver were consistently found only in English sole and starry flounder from the urban-associated areas.

2. In general, lesions in organs other than the liver failed to show higher prevalences in the urban-associated estuaries compared to the reference estuary (McAllister Creek). Individuals of the two flatfish species with certain lesions of the liver (other than above), gills, and kidney, as well as lesions of other tissues (skin, gastrointestinal tract), were found in the urban estuaries as well as in the reference estuary. The widely occurring lesions of the liver were fatty change and hepatocellular necrosis in both species, and hemosiderosis in English sole and blood cysts in starry flounder. English sole with the gill lesion, respiratory epithelial hyperplasia, were found only in urban estuaries; however, starry flounder with this lesion were equally or more prevalent in the reference estuary. Widely observed abnormalities of the skin and gastrointestinal tract were chronic dermal necrosis and parasitic infections, respectively.

3. Most of the lesions observed in this study probably were the result of long-term exposure to one or more environmental factors. The length of exposure time is suggested by the average age of English sole with liver lesions, 4 to 6 years. However, certain idiopathic liver lesions were detected in fish as young as one year of age.

4. The prevalence of English sole with two types of liver lesions, megalocytic hepatosis and hepatocellular eosinophilic hypertrophy, was significantly higher ($p<0.05$) in January, 1980 (24.3 and 14.8%, respectively) than in January, 1979 (14.8 and 9.3%, respectively). Whether this increase represents a seasonal fluctuation in prevalence or an overall increase in prevalence, suggesting a worsening of the health of English sole, is not presently known.

5. The abundance of English sole and starry flounder in the Duwamish Waterway and McAllister Creek was seasonally variable and, in the case of the Waterway, varied according to the distance of the sampling stations from the mouth of the Waterway. The abundance of English sole in both estuaries was lowest during the spring, and in the Waterway the abundance was similar during the other three seasons. For starry flounder, the abundance in the Duwamish Waterway was lowest during the initial fall, winter, and spring sample collections, and 4 to 14 times higher in the summer and succeeding winter collections, respectively. In the Waterway, English sole tended to be most abundant near the mouth during the winter, when flow was highest, and near the turning-basin (the upper portion of the sampling area) during the summer and fall, when flow was lowest. A similar trend was observed for starry flounder, except that, since this species prefers or tolerates relatively low-salinity water, they were generally not collected at stations at the mouth of the Waterway.

6. The concentrations of AHs and metals in sediment samples from the Lake Washington Ship Canal and the Duwamish Waterway were similar; however, the concentration of PCBs in sediment from the Lake Washington Ship Canal was considerably lower than in sediment from the Duwamish Waterway. None of the AHs or PCBs measured in sediments from these two urban estuaries were detected in sediment from the reference estuary, McAllister Creek, while sediments from the Snohomish River had levels of AHs and PCBs intermediate between the two types of estuaries. Concentrations of toxic metals (cadmium, mercury, chromium, lead, and copper) were generally considerably higher in sediment from Duwamish Waterway and Lake Washington Ship Canal than from McAllister Creek. With the exception of cadmium, which was detected only in these two urban estuaries, the levels of the other metals were 2 to 20 times lower in the reference estuary.

7. The concentrations of PCBs and metals in tissues of flatfish reflected the levels found in sediment samples collected from areas in which the fish were captured. Although most AHs were not detected in liver tissue of fish from any of the sampling areas, low concentrations of naphthalene were routinely found in tissues of fish from the Lake Washington Ship Canal and Duwamish Waterway. The almost complete absence of AHs in flatfish tissue was almost surely due to the ability of flatfish to metabolize AHs. The detection of naphthalene is consistent with laboratory findings that flatfish do not metabolize this compound as rapidly as higher molecular weight AHs.

8. The laboratory studies in which English sole were exposed to chemical contaminants found in sediments from the Duwamish Waterway did not induce pathological conditions observed in English sole from the Duwamish Waterway. However, the experiments did demonstrate that extracts of sediments from the Waterway have certain components that are toxic to sole that were not present in reference sediments. Other experiments clearly showed that BaP, a carcinogenic AH present in sediment from the Duwamish Waterway, was readily taken up directly from contaminated sediment by English sole.

SECTION 3

RECOMMENDATIONS

The results of the field survey portion of this study demonstrate that any comprehensive monitoring program intended to evaluate the health of aquatic animals should include histopathology as well as other scientific disciplines, such as analytical chemistry and ecology. The histopathological procedures and diagnostic criteria should also incorporate the standards employed by veterinary pathologists and toxicologists.

Additional research efforts need to be expended on elucidating the causes of the lesions observed in flatfish in the Seattle waterways. The laboratory studies reported here provide preliminary evidence that sediment in the Duwamish Waterway contains factors which are toxic to English sole. Further experimentation utilizing both controlled laboratory exposures and semi-controlled field experiments will be needed to verify these preliminary observations and to characterize the nature of the etiological factors. The laboratory experiments could involve long-term exposures of juvenile flatfish to various fractions of sediment extracts, administered orally or by injection. Field experiments could employ long-term *in situ* exposures of flatfish (held in cages) to bottom sediment in contaminated and uncontaminated estuaries.

An important question left unanswered by this study was the effect of the observed diseases in English sole and starry flounder on the survival of these two species. In order to provide further information for answering this basic, key question, a complex ecological investigation, including tagging studies, should be performed. Alternatively or additionally, flatfish could be captured from selected areas, biopsied and pathological analysis of liver tissue performed, and transferred to holding facilities in the laboratory where survivial could be monitored.

SECTION 4

EXPERIMENTATION

METHODS AND MATERIALS

FIELD STUDIES

Sampling Areas and Sampling Stations

The four areas of Puget Sound which were sampled in this study were: (1) the Duwamish Waterway in Seattle, Washington (seven stations, A-G, Figs. 1 and 2); (2) the Lake Washington Ship Canal in Seattle, Washington below the Hiram M. Chittenden Locks (one station, Fig. 1); (3) the Snohomish River estuary near Everett, Washington (two stations, Fig. 1); and (4) McAllister Creek which enters into the Nisqually delta about twenty miles south of Tacoma, Washington (two stations, Fig. 1). Since the first three areas sampled are within or close to urban industrial centers, they are termed "urban-associated estuaries". The fourth area served as a reference area due to its distance from urban and/or industrial influences.

Target fish species (English sole and starry flounder) were collected by an otter trawl with the following specifications: 7.5 m opening, 10.8 m total length, 3.8 cm mesh in the cod end. Individual trawls at each station were of 5 minutes duration at approximately 2.5 knots, sweeping approximately 0.2 nautical miles. Sediments were collected with a modified Van Veen sediment grab (Word 1976). Temperature, salinity and dissolved oxygen measurements at or near the bottom at each sampling station were taken with a Hydro-lab.[1] The seasons and areas sampled during this study are described in Table 1.

Examination of Fish and Necropsy Procedures

At each station, after collection by otter trawl and separation by species, the two target species were placed in holding tanks containing fresh seawater. All fish were examined for externally visible abnormalities. The final composition of the necropsy subsample for each station consisted of up to 30 fish of each species. To gain a broad size distribution of fish within the subsamples, the intent was to select randomly equal numbers of fish for necropsy from three length groups: <10 cm, 10-20 cm, and >20 cm.

Fish to be necropsied were measured for total length (to the nearest mm), weighed (to the nearest g), and assigned an individual field number. The spinal cord was then severed and otoliths were collected from each fish for aging purposes. The gonads were examined and the

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[1] Use of brand or trade names of materials used in this study does not constitute official endorsement by the National Marine Fisheries Service.

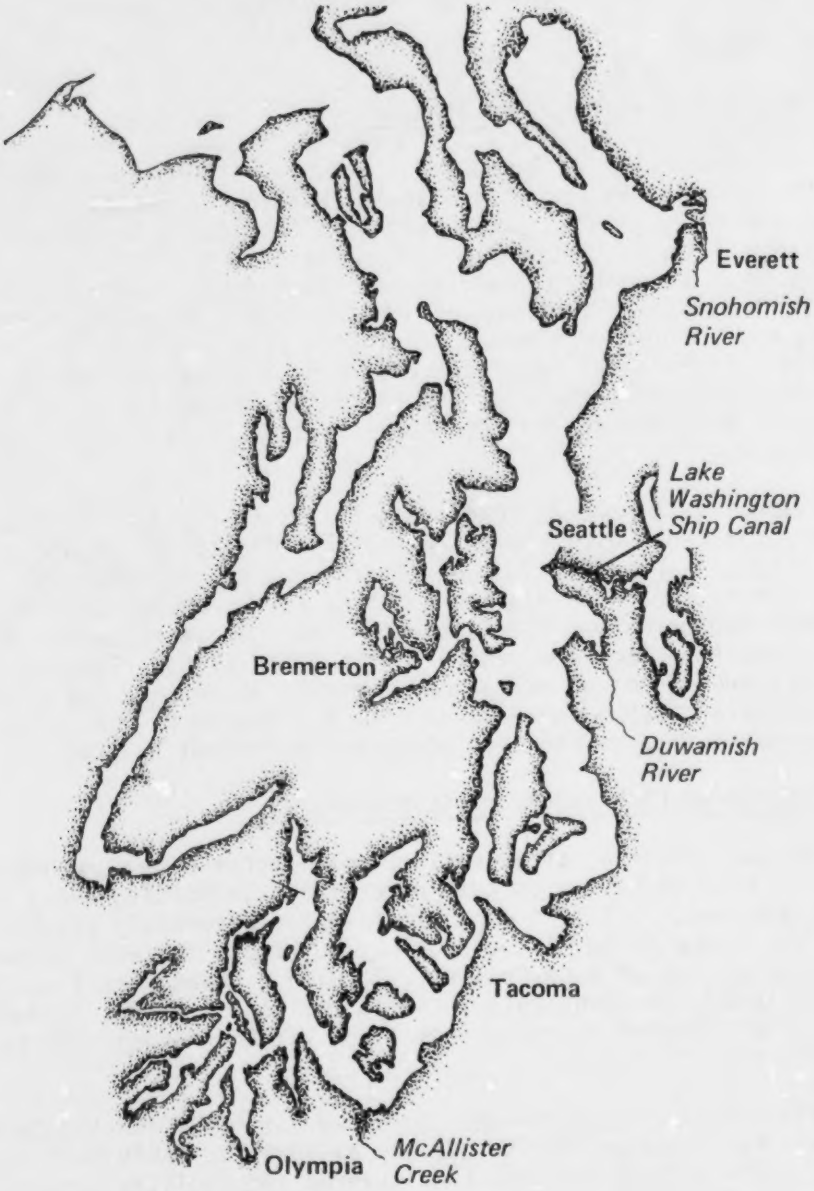
Everett
Snohomish River
Lake Washington Ship Canal
Seattle
Bremerton
Duwamish River
Tacoma
Olympia
McAllister Creek

FIGURE 1. General map showing the four field sampling areas in Puget Sound (Snohomish River, Lake Washington Ship Canal, Duwamish River Waterway and McAllister Creek).

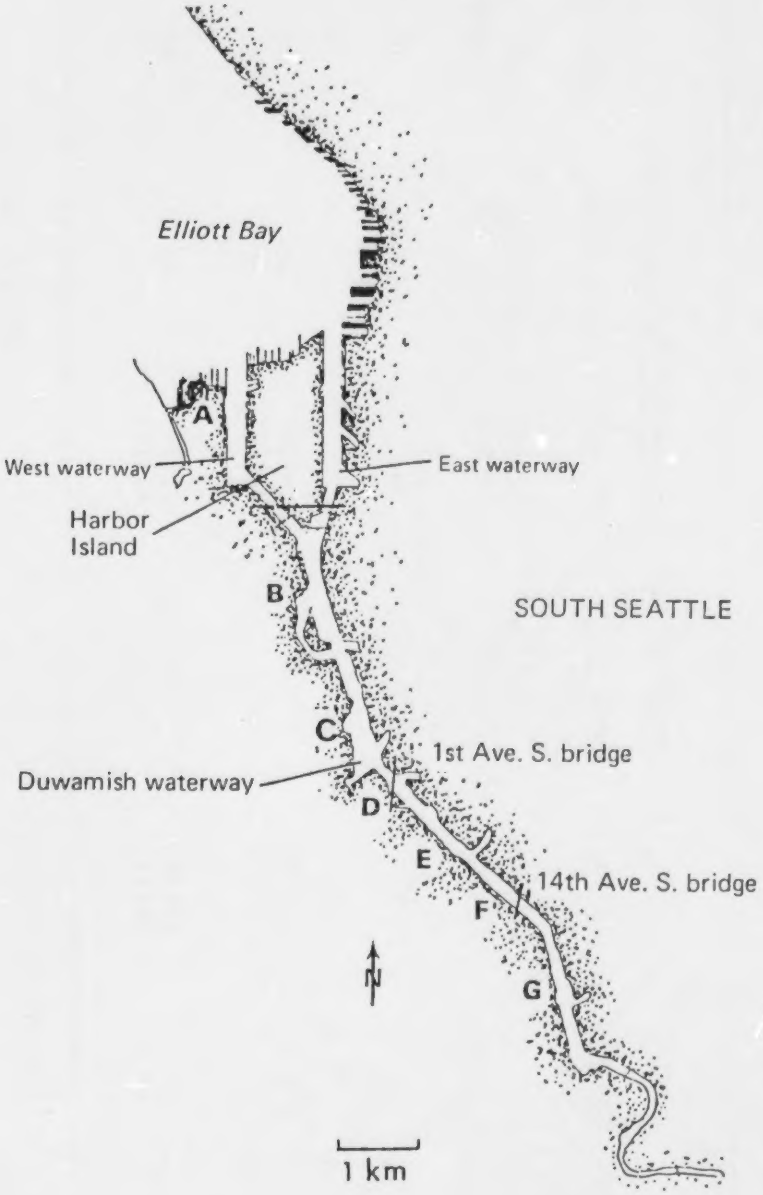
Elliott Bay
A
West waterway
East waterway
Harbor Island
B
SOUTH SEATTLE
C
1st Ave. S. bridge
Duwamish waterway
D
E
14th Ave. S. bridge
F
G
N
1 km

FIGURE 2. Detailed map of field sampling stations in the Duwamish Waterway system.

TABLE 1. English sole and starry flounder which were examined for externally visible and microscopic abnormalities.

Estuary	Species[a]	Number of Fish Examined For Each Sampling Oct. 1978	Jan. 1979	Apr. 1979	July 1979	Jan. 1980
Duwamish Waterway	ES	264(168)[b]	178(93)	127(95)	311(121)	209 (94)
	SF	42 (27)	52(51)	90(90)	76 (26)	187(103)
Lake Washington Ship Canal	ES	NS[c]	50(31)	NS	44(17)	NS
	SF		2 (2)		0	
Snohomish River	ES	24(24)	NS	19(19)	NS	NS
	SF	1 (1)		(8)(8)		
McAllister Creek	ES	45(18)	NS	22(19)	NS	NS
	SF	26(16)		41(26)		

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[a] ES, English sole; SF, starry flounder.

[b] Number in parenthesis is the number of fish which were examined for external abnormalities, necropsied, and examined for histopathology; numbers not in parenthesis were examined only for externally visible abnormalities.

[c] NS, not sampled.

sex was determined. Tissues were excised from the following organs: liver, spleen, gastrointestinal tract, gall bladder, gills, kidney, heart, gonads, skin, and fins. (Skin and fins were collected only when gross lesions were present.) These tissues were placed in cassettes individually labeled with the appropriate field number and placed immediately in Dietrich's fixative (Malins et al. 1980). When a grossly visible lesion was observed in any organ, a portion of that tissue with the surrounding normal-appearing tissue was excised and fixed with the other organs described above.

Histological Procedures

Tissue specimens fixed in Dietrich's were removed from the fixative after a minimum of 48-hours fixation time, processed automatically with an Autotechnicon tissue processor, embedded in paraffin with a Tissue Tek embedding center, and sectioned at 5 microns (Preece 1972). Tissues such as those of gills and fins were decalcified following fixation, using a commercial decalcification solution (Scientific Products, Redmond, WA) prior to tissue processing with the Autotechnicon. Sections were routinely stained with Mayer's hematoxylin and eosin-phloxine (Luna 1968). All stained tissues from each fish were examined by light microscopy, and descriptions of any observed lesions and/or parasites were recorded. When necessary for further characterization of lesions, additional sections were cut and stained with special stains (Thompson 1966, Luna 1968, Preece 1972).

Histopathological Procedures

All slides were examined employing a "blind" system. As mentioned previously, each fish was first assigned a field number prior to necropsy. After processing, embedding, and sectioning were completed, and prior to microscopic examination, each fish was assigned an identification number derived from a random number table. Therefore, examining histopathologists were given information on the species, length, weight, sex, and presence of gross pathology, but did not know the area or station of capture. Sections with microscopic lesions were classified using standard nomenclature.

Statistical Methods

The G-statistic was used to test the null hypothesis that the fish lesion frequency within a given area or at a particular station did not differ from the average lesion frequency (for the particular lesion being analyzed) for all areas sampled. In other cases, the G-statistic was also employed to assess the statistical significance of lesion frequency variations between areas and different seasonal samplings. Since the data consisted usually of two categories (fish with lesions vs. normal fish, or two lesion frequencies being compared), the Yates correction for continuity (Sokal and Rohlf 1969) was used. This method takes into account both the number of fish examined and the number of lesions observed.

Chi-square tests were also employed in evaluating the independence of two variables (Zar 1974). This analytical technique was useful in assessing the degree of association between specific pathologic conditions in a given species of fish.

Chemical Analyses

Samples of liver tissue from English sole and starry flounder, and of bottom sediments were analyzed for AHs, PCBs, and metals. In addition, liver tissues were analyzed for percent lipid.

Samples of liver tissue, collected as part of the necropsy procedure, were placed in methylene chloride-rinsed glass vials, immediately frozen, and kept in frozen storage until they were analyzed. The analytical procedures for organic compounds involved tissue homogenization, tissue extraction with organic solvents, and fractionation of the extracts using column chromatography. Appropriate fractions were analyzed using glass capillary gas chromatography employing flame ionization, electron capture, or mass spectrometer detectors (Brown et al. 1980, Ramos and Prohaska 1981). Analyses for metals were performed by plasma emission spectroscopy as in Malins et al. (1980).

Five sediment sample cores (5.5 cm diameter x 2 cm height) were collected from each of two grab samples at each sampling station. The ten cores of sediment were thoroughly mixed in a Teflon beaker with a Teflon stirring rod. Portions of the mixed sediment were placed in a glass bottle for analysis of organic compounds and in a plastic container for metals analysis. These samples were frozen in the field and kept frozen until they were thawed for analysis. Analyses for metals were performed in essentially the same manner as for liver tissue. Sediment samples were also analyzed for total organic carbon and grain size distribution. Sediment grain size was measured by screening samples of sediment through a series of standard sieves according to methods reported by Krumbein and Pettijohn (1938). Total organic carbon was measured using a LICO (manufacturer) induction furnace.

LABORATORY STUDIES

Several laboratory experiments were performed to attempt to define the possible relationship between pollutants present in the Duwamish Waterway and various lesions observed in English sole from this area. In some tests, sole were held in aquaria containing sediment from either the Duwamish Waterway or two relatively unpolluted areas, Port Madison and the Snohomish River. Sole were also exposed to extracts of sediment and interstitial water from the Duwamish Waterway and Port Madison by intraperitoneal (i.p.) injection. As a means of evaluating if the aromatic hydrocarbons present in the sediment extracts were bioavailable to sole by natural means, sole were exposed to reference sediment contaminated with radiolabeled BaP. The uptake and fate of BaP was determined.

Care of Experimental Fish

English sole collected by beach seine or bottom trawl from non-urban areas of Puget Sound (principally Mutiny Bay, Whidbey Island) were used for all experiments. The average lengths and weights of the fish used in each experiment are shown in Table 2. Newly captured fish were acclimated in holding tanks for at least 3 days prior to experimentation. They were maintained in all-glass aquaria supplied with running seawater. Water temperature during the experimentation period ranged from 8.5°C to 12.4°C.

Fish used in the effects studies were fed clams dug from non-urban beaches of Puget Sound and frozen until use, frozen euphausids taken from Alaskan waters and obtained from a commercial supplier, and earthworms from local suppliers. The fish were fed ad libitum, and the food supply was replenished twice daily.

English sole used in the bioavailability experiment were kept in flowing seawater (salinity of 28% and 13.0+0.5°C) and fed a diet of minced clams, obtained as above, for 2 weeks. The feeding was stopped 3 days prior to the initiation of the experiment.

Exposures to Sediment Extracts

Preparation of extracts of sediment and interstitial water--

Duwamish Waterway sediment and Port Madison sediment were extracted using identical procedures. Briefly, the sediment extraction involved the following procedure: (1) separating the sediment from associated water by centrifugation; (2) extracting the sediment with methanol; (3) extracting the sediment with a 2:1 mixture of the sediment with a 2:1 mixture of methylene chloride and methanol; (4) pooling the extracts and extracting the methanol-pooled extract with saline (2%); and (5) storing at 4°C the resulting methanol-aqueous phase and concentrating the methylene chloride phase by evaporation. This bulk concentrate was dissolved in corn oil, the remaining methylene chloride was evaporated, and the residual, undiluted material was injected with a corn oil vehicle into English sole. The interstitial water was extracted in a similar manner.

The chemical composition of the original sediments is shown in Table 3.

Exposure of fish to sediment extracts--

Two experiments involving i.p. injection of sediment extracts were completed. Fish were held in 30 x 50 x 30 cm aquaria. In Experiment One, for each type of extract, i.e., Duwamish Waterway (test) and Port Madison (reference), three dilutions (1/2, 1/20 and 1/200) of sediment extract in corn oil were each injected (0.1 ml per fish) into one of

TABLE 2. Lengths and weights of English sole used in laboratory experiments.

Experiment	Number of Specimens	Mean Length (mm)	Length Range (mm)	Mean Weight (g)	Weight Range (g)
Sediment Extract Injection					
Experiment One	21	103	87-118	10.4	5.8-15.0
Experiment Two	39	110	78-143	10.3	4.8-18.6
Interstitial Water Injection					
Experiment One	20	121	94-173	13.0	5.6-37.8
Sediment Contact					
Experiment One	200	103	73-132	9.8	3.4-19.6
Experiment Two	67	130	91-175	18.1	5.3-40.3
Bioavailability					
Experiment One	3	210	200-220	70.0	65-76

TABLE 3. Concentrations of selected compounds in sediment from the Duwamish Waterway, the Snohomish River, and Port Madison used in laboratory experiments.

Compounds	Snohomish River	Port Madison	Duwamish Waterway
		(ng/g dry wt.)	
Fluorene	49	5	95
Dibenzothiophene	43	7	110
Phenanthrene	250	35	510
Anthracene	22	4	94
Fluoranthene	450	57	1200
Pyrene	330	63	1000
Benz[a]anthracene	150	44	1000
Chrysene	150	30	670
Benzo[e]pyrene	43	33	580
Benzo[a]pyrene	26	24	650
Total PCBs	71	56	713
Total chlorinated butadienes	1	21	4
p,p'-DDD/o,p-DDT	3.5	1	11
p,p'-DDE	<1	<1	5

three groups of three fish each. An additional group of three fish received corn oil alone. Fourteen days following injection, surviving fish were sacrificed and necropsied. The necropsy procedure was similar to that described for the field studies, except that blood was collected by syringe from the caudal vessels just ventral to the vertebral column, or in smaller fish by excising the caudal fin. Aliquots of whole blood were used immediately after collection to determine hemoglobin concentrations and hematocrit values.

In Experiment Two, fish were injected with extracts of Duwamish Waterway and Port Madison sediments which were approximately three times more concentrated than those used in Experiment One. For each type of sediment extract, each of three dilutions (undiluted, 1/2 and 1/10) were injected (0.1 ml per fish) into one of three groups of five fish each. Control groups of five fish each were injected with corn oil alone (vehicle control) or received no injection (untreated control). Eighteen days after the first injection, surviving fish in each group were reinjected with the same amount and dilution of the test or reference substance as described for the first injection. Survivors were sacrificed and necropsied five days after the second injection.

One experiment examining the effects of the injection of interstitial water was performed. In this test, groups of five fish were injected i.p. (0.1 ml per fish) with extracts of either Duwamish Waterway or Port Madison interstitial water (undiluted and diluted 1/2 in corn oil). Surviving fish were sacrificed and necropsied 16 days after injection.

Exposures to Bottom Sediments

Two experiments were performed in which fish were maintained for 2 or 3 months in aquaria containing test sediment (Duwamish Waterway) or reference sediment (Snohomish River or Port Madison). The chemical composition of each sediment is shown in Table 3.

In Experiment One, English sole were maintained for 92 days in 30 x 90 x 120 cm aquaria containing 3 cm of Duwamish Waterway or Snohomish River sediment. Six fish were necropsied and examined hematologically and histopathologically at the beginning of the experiment for comparison with fish sampled at later times. One hundred fish were maintained on each sediment type; each group (test or reference) was divided equally between two aquaria. Fish were sacrificed and necropsied from each group on days 29, 57 and 92.

In Experiment Two, English sole were maintained under conditions similar to those described for Experiment One, except that the reference sediment was from Port Madison. At the time the experiment was started (0-time), five fish were necropsied for histopathological and hematological examination. Thirty-five fish were placed on Duwamish River sediment and 32 were placed on Port Madison sediment. Three fish were sacrificed and necropsied from each group on day 37 and five were sacrificed and necropsied from each group on day 65.

Statistical Analysis of Effects Data

Standard statistical tests (Zar 1974) were used for analysis of mortality, lesion frequency and hematological data. Mortality data for the various treatment groups were arranged in 2 x 2 contingency tables and a chi-square statistic was calculated for each table to determine whether the distribution of deaths in a given experiment was independent of the type of treatment administered. Similar tests were performed on the histopathological data to ascertain whether the frequency of microscopically-observed anomalies was independent of treatment type.

One-way analysis of variance and the Neuman-Keuls multiple range test were employed for comparisons of mean hematocrit or hemoglobin values among the various test and control groups in the experiments. Apparent trends in hematological data were further investigated using simple linear regression.

Exposure of English Sole to Aromatic Hydrocarbons in Sediment

Sediment collected from a reference area (Port Susan) was mixed with [^{3}H] BaP [3 mg (5480 µc) per 2 l of sediment]. Mixing was performed by first dissolving the BaP in acetone, adding this solution to 0.5 l of sediment, evaporating the acetone with a stream of nitrogen, and combining and mixing the contaminated sediment with the remaining 1.5 l of sediment. The [^{3}H] BaP-sediment was placed in glass aquaria (17.0 l capacity), seawater was added at a flow rate of 20 l/day for 1 day. Three fish (see Table 2 for size data) were then placed in the experimental tank and sampled 24 h after initiation of exposure.

Analysis of sediment and sediment-associated water--

Samples of sediment and sediment-associated water (SAW) were taken from 2 cm below the sediment/water interface. The wide end of a glass pipette was vertically inserted into the sediment while the tip of the pipette was covered. After positioning the pipette, the tip was uncovered to allow sediment and SAW to rise within the pipette. The sample was carefully transferred to a vial and the SAW was decanted after the suspended particles had settled. Samples of water from near the air/water interface were also taken.

Samples of wet sediment and 1-ml aliquots of unfiltered SAW were analyzed for total radioactivity [^{3}H] using liquid scintillation spectrometry as described by Varanasi et al. (1978, 1979).

Analysis of tissues--

Fish were killed by a blow to the head and samples of blood, liver, muscle, and bile were analyzed for [^{3}H] using methods detailed by Varanasi and Gmur (1981a). Enzymatic hydrolysis of the conjugated metabolites of BaP in the bile samples were analyzed for glucuronides using Glucurase (Sigma Chemical Co., 500U per assay).

RESULTS AND DISCUSSION

FIELD STUDIES

The field survey portion of this investigation was primarily intended to document the prevalence, geographical distribution and appearance of lesions in English sole and starry flounder from selected estuaries in Puget Sound. As part of this effort, the catch data and length/weight/age characteristics of these two species were also obtained. Chemical analyses were performed on samples of sediment and fish liver tissue.

Of the 1293 English sole examined for externally visible lesions, 673 were sacrificed, necropsied, and examined for microscopic lesions in major organs. In the same manner, 525 starry flounder were examined externally and 350 were necropsied. Two major types of lesions were observed, parasitic and idiopathic (lesions of unknown etiology). The description of these lesions which follows will be divided according to species and subdivided into affected organs.

Pathological Conditions of English Sole

Lesions of the skin and fins--

A broad spectrum of parasitic and idiopathic lesions were diagnosed in English sole skin and fin sections. Several could be diagnosed grossly, but some could only be diagnosed definitively at the light microscopic level. Because skin and fin sections were taken only from fish with grossly visible anomalies, only the prevalence of fish with these anomalies will be presented. Those lesions which were detectable only by microscopic examination will be described, but prevalance data will not be given.

Parasitic lesions--The most common parasitic condition was readily visible on gross inspection, but prevalence data for this condition was not compiled. In this parasitic infection, members of the nematode genus Philometra were grossly visible in the subcutaneous tissue of the skin and fins, and often parasitized the peritoneum, peritoneal cavity, kidney, pericardial cavity, and body musculature. The typical host response to these blood-feeding nematodes was chronic inflammation with fibrosis. Microscopic examination showed that most of the parasites observed were females bearing filariae. Free filariae were often found within the subcutaneous tissue. Affected English sole were found in all sampling areas.

Another helminth infection was grossly visible as small 3-4 mm subcutaneous nodules in the skin and fins. A definitive diagnosis was possible only by microscopic examination. This infection of the dermis by digenean trematode metacercariae was accompanied by dermal fibrosis, chronic inflammation, and necrosis of the dermal connective tissue elements. The metacercariae were miniature adults lacking only a developed reproductive system, and are presumably infective to the definitive host (Chitwood and Lichtenfels 1972). English sole are, therefore, very likely an intermediate host. Each metacercaria was surrounded by a cyst, thought to be elaborated principally by the parasite. However, in most sections there was significant fibrotic host reaction as well, with occasional congestion of the subcutaneous vasculature. Precise identification to family or genus was not attempted for these digenean trematode metacercariae, but morphologic similarity between cases indicates that a single genus was represented.

Skin metacercarial lesions were detected microscopically in a few fish (10) from the Lake Washington Ship Canal, Duwamish River and Snohomish River. No cases were found in English sole from McAllister Creek.

Viral lesions--A single case of a dermal viral infection (lymphocystis virus disease) was detected in an English sole from the Duwamish Waterway. This condition is ubiquitous worldwide, and has to date been reported from northwestern and western coastal marine waters in the U.S. (Alpers et al. 1977a, McCain et al. 1978a, 1979, McCosker and Nigrelli 1971). To our knowledge, this is the first report of lymphocystis virus disease in Puget Sound, and the first report in English sole (Parophrys vetulus). The light and electron microscopic features of this lesion are described in Figures 3 to 5. (For a more detailed description of this lesion, see Appendix 1.)

Skin tumors--Tumorous skin lesions were also infrequently detected grossly in English sole. These tumors were of three main types, termed angioepithelial nodules (AEN), transitional angioepithelial nodules (TAEN) and epidermal papillomas (EP). All three are elements of a single disease complex common in pleuronectids (McArn et al. 1968), the etiology of which is controversial, but is thought to be caused by a parasitic amoeba (Dawe 1980, Myers 1981). The tumors are known to progress from AEN to TAEN to EP. These lesions are described in detail in Appendix 1.

AEN lesions were detected in all four sampling areas, with the highest prevalence in McAllister Creek (10.3%, 4 of 39 fish) and the lowest in the Duwamish River (0.2%, 1 of 565 fish). The prevalence in Lake Washington Ship Canal was 4.7% (2 of 48 fish), and in the Snohomish River the prevalence was 2.7%. Transitional tumors were found only at McAllister Creek (5.1%, 2 of 39 fish) and the Duwamish Waterway, (0.2%, 1 of 565 fish), and the single case of a well-developed EP was from the Duwamish Waterway.

Fin erosion--An idiopathic fin lesion, termed fin erosion (Wellings et al. 1976b) was visible grossly as a loss, distortion or fusion of fin rays accompanied by hemorrhage and fibrogranulation tissue on the fin surfaces and at the juncture of the fin rays and body musculature. The condition was most common on the anal and dorsal fins. The free margins of damaged fins were often ulcerated, and scarification of fin tissue

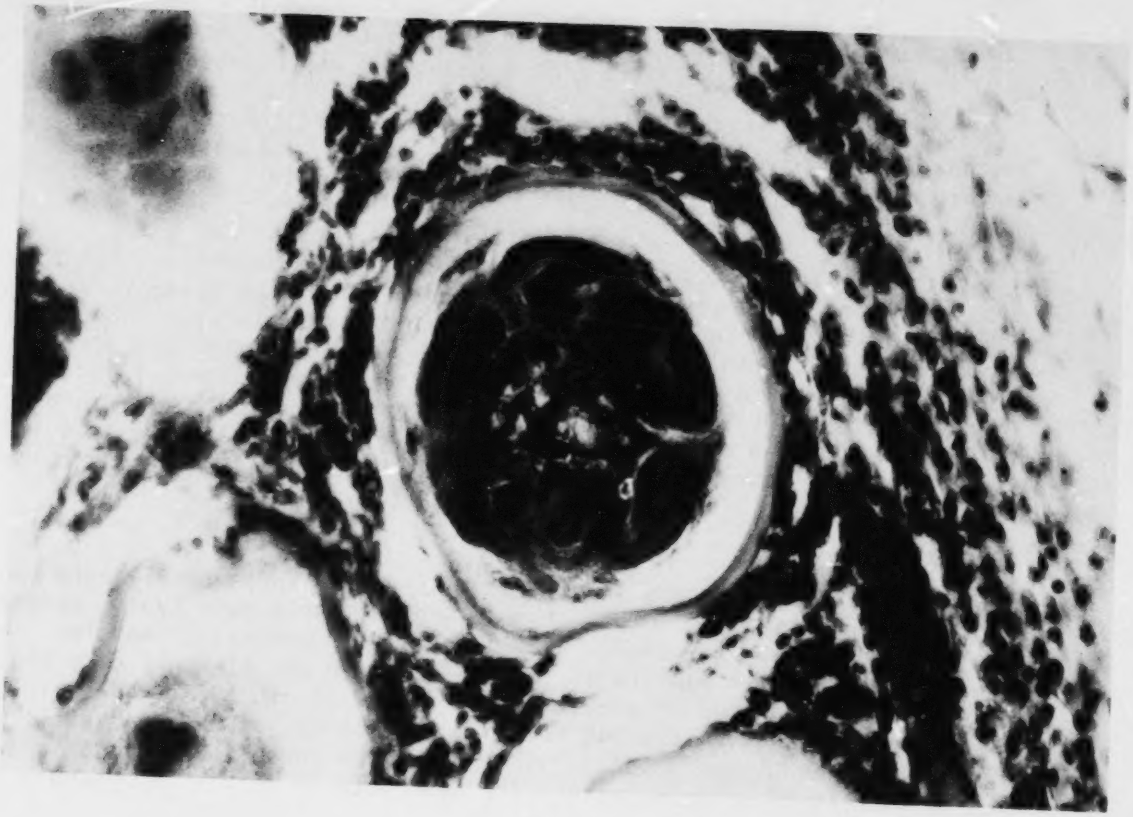

FIGURE 3. Micrograph of lymphocystis skin lesion in an English sole. In the center is a lymphocystis-infected cell with the characteristic enlarged nucleus, densely basophilic cytoplasmic inclusion bodies and external hyaline capsule. H & E, 975X.

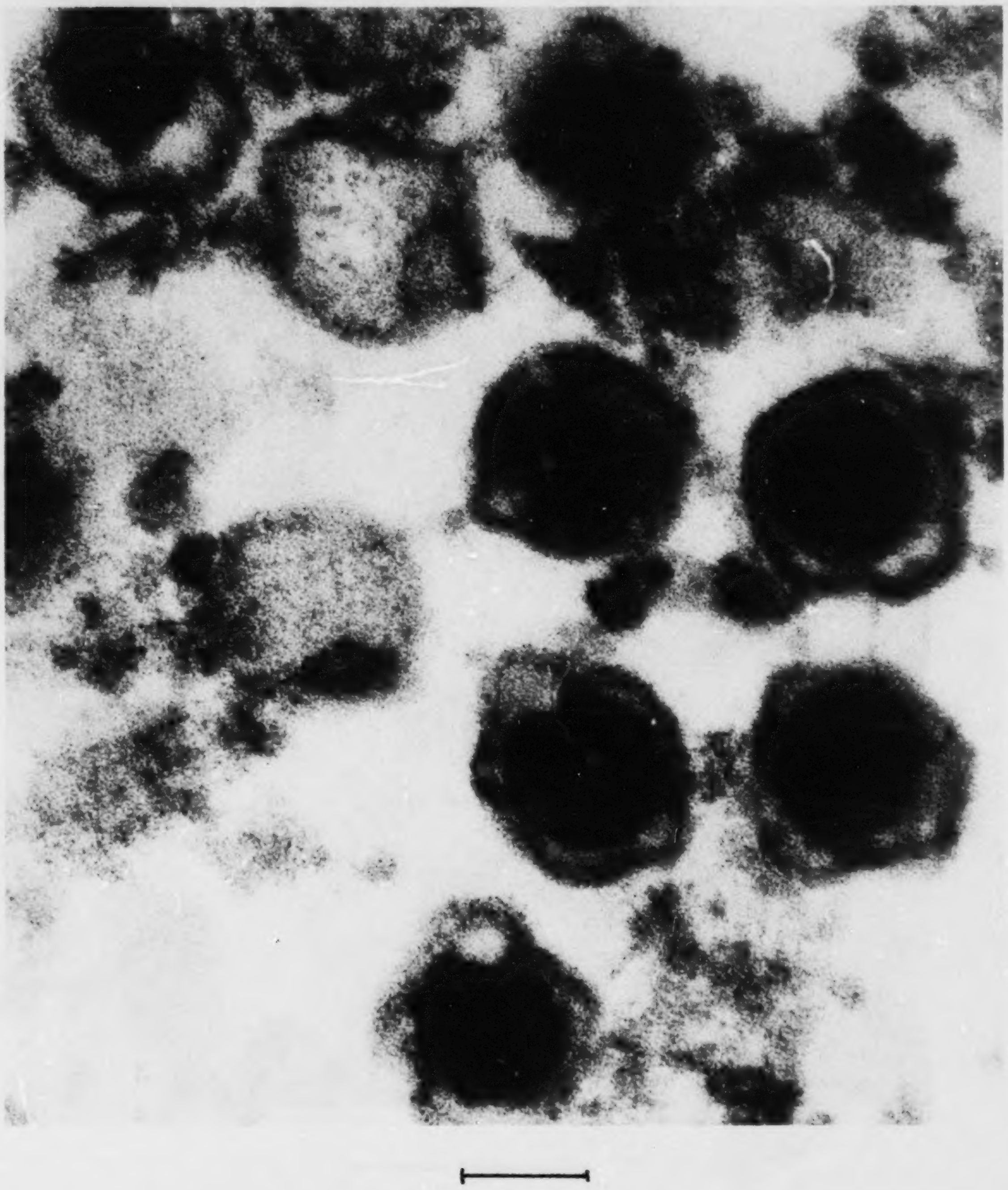

FIGURE 4. Electronmicrograph of intracytoplasmic lymphocystis virus particles. The particles have an internal nucleoid structure and outer capsids.

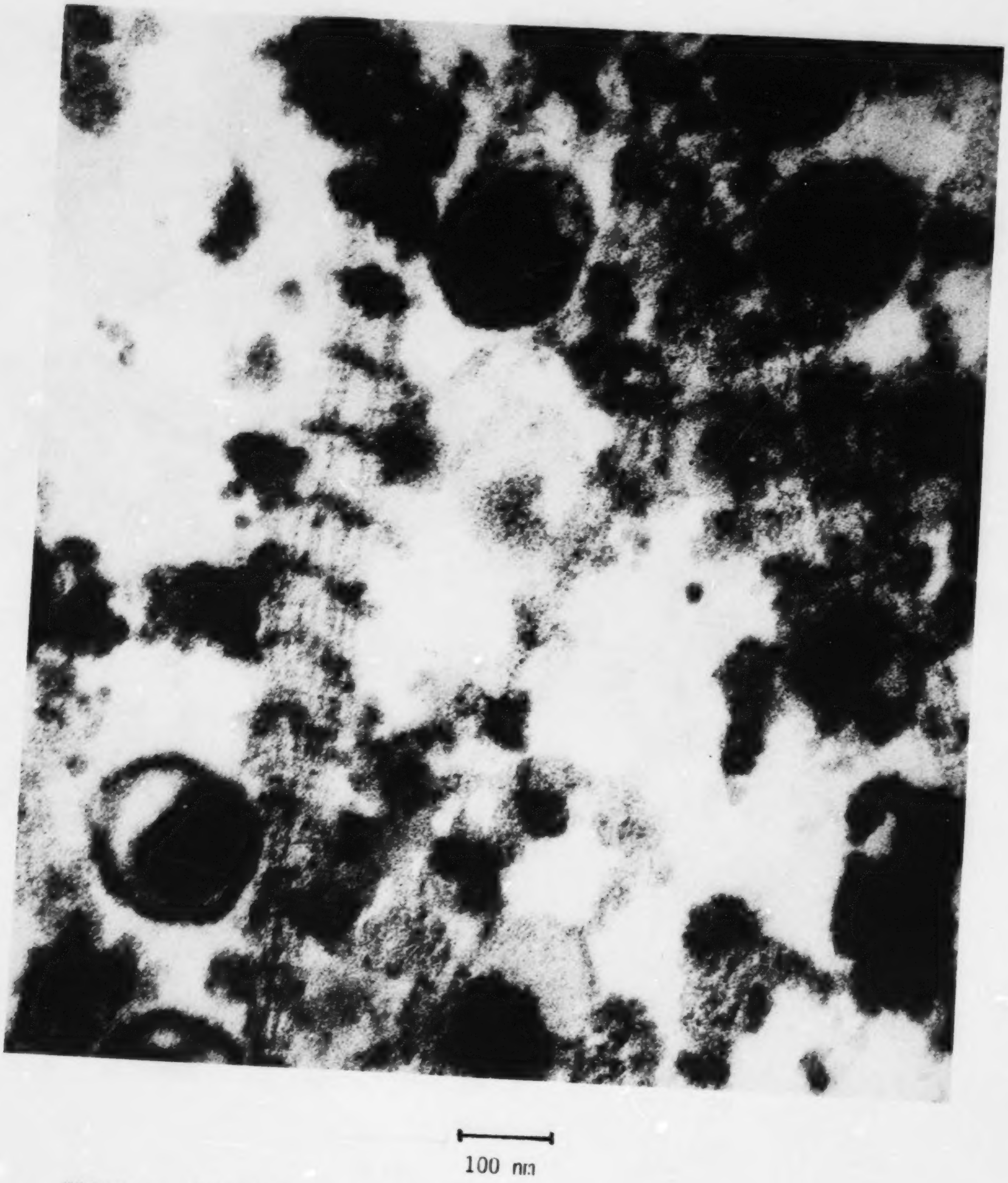

FIGURE 5. Electromicrograph of intracytoplasmic lymphocystis virus particles and fibrillar structures showing banding periodicity.

was common, with folding and fusion of adjacent fin rays. In the most severe cases, the fin rays were entirely absent and the residual tissue was scarred, retracted, and lacked rigidity.

The microscopic characterisitcs of these lesions have been previously described in detail (Wellings et al. 1976b), but the more salient features will be described here. The condition is generally a chronic fibrosing disease with epidermal hyperplasia, dermal fibroplasia and fibrosis, infiltration of mononuclear inflammatory cells, increased numbers of melanophores in the dermis, blood vessel sclerosis, and increased epidermal eosinophilic granular cells. The hallmark of this condition is resorption of the bony fin rays and replacement with fibroblasts and collagen. Generally, this constellation of features could be found in any fully developed case of fin erosion. Occasional ulceration of the epidermis was present, and inclusion cysts lined by epidermal cells were often observed in more severe cases. Pathogenic bacteria were not detected in the Brown and Brenner Gram Stain (Luna 1968), nor were other potential protozoan agents. Viral inclusions were not detected at the light microscope level, and the condition was not commonly associated with any parasitic infection.

Two microscopically definitive cases (0.4%, 2 of 565 fish) of this condition in English sole were detected in the Duwamish Waterway, and in none of the remaining sampling areas. Fin erosion is much more common in starry flounder from the Duwamish River, but the lesion is identical and prevalence data will be presented in the section describing skin and fin lesions in starry flounder.

Miscellaneous skin lesions--Other lesions found in the areas sampled were as follows: non-specific epidermal necrosis and spongiosis, dermal chronic inflammation, exostosis of fin rays (found in only one fish from the Duwamish Waterway), dermal necrosis, subcutaneous hemorrhage, epidermal hyperplasia without true fin erosion, subcutaneous edema, and the presence of dermal nematode filariae.

Liver lesions--

Both idiopathic and parasitic lesions were observed in the livers of English sole. The major idiopathic liver lesions included megalocytic hepatosis (MH), hepatocellular regeneration (HR), hepatocellular eosinophilic hypertrophy (HEH), minimum deviation nodule (MDN), hepatocellular nodular hyperplasia (HNH), liver cell adenoma (LCA), hepatocellular carcinoma (HC), cholangiocellular carcinoma (CC), mixed carcinomas (MC), cholangiofibrosis (CF), hepatocellular necrosis (HN), fatty change (FC), hemosiderosis (HEM) and hemangiomas. The only parasites consistently associated with lesions of the liver were members of the sporozoan genus Myxidium (Butschli), family Myxidiidae (Thelohan). The morphologic characteristics of these lesions will first be presented in this section, followed by a section dealing with the geographical distribution and prevalence of the lesions.

Megalocytic hepatosis (MH)--Megalocytic hepatosis was characterized by massive increases in the diameters of hepatocytes and their nuclei (i.e., megalocytosis) as a non-inflammatory lesion of the liver, i.e., hepatosis (Jones and Butler 1975). Other characteristics included cytoplasmic acidophilia or a "ground-glass appearance" with occasional fibrillar "fingerprint" cytoplasmic structures characteristic of proliferation of the smooth endoplasmic reticulum (Bannasch 1976), and nuclear hyperchromasia. The enlarged nuclei were generally highly vesicular and exhibited aberrent, dense distributions of chromatin, often marginated, indicating an elevation in DNA content (Fig. 6). The cytoplasm commonly showed signs of degeneration, including hydropic degeneration and hyalinization. Excessive amounts of cytoplasmic iron-containing pigment (hemosiderin) were commonly found in the affected megalocytes by the Prussian Blue reaction (Preece 1972). No proliferation of megalocytic hepatocytes was seen.

The morphology of this lesion closely parallels hepatotoxic changes induced in fish and other vertebrate species by diverse types of naturally occurring and xenobiotic chemical species. Exposure to pyrrolizidine alkaloids (plant extracts) produces hepatic megalocytosis in mammals (Jubb and Kennedy 1970, McLean 1970, Jago 1969, McLean 1974), as do cyclopropenoid fatty acids in fish (Sinnhuber et al. 1968, Struthers et al. 1975, Malevski et al. 1974, Hendricks et al. 1980), PCBs in mammals and fish (Koller and Zinkl 1973, Nishizumi 1970, Hinton et al. 1978, Kimbrough and Linder 1974), and phenobarbitone in mice (Jones and Butler 1975).

The nuclear characteristics in MH suggest action by a mitotic poison, resulting in polyploid hepatocytes which fail to divide. The degenerative cytoplasmic changes suggest parallel action by a cytotoxin. Quite possibly, megalocytic hepatosis is a subacute or chronic manifestation of the cytotoxicity of hepatotoxin(s) and/or hepatocarcinogen(s). In the early stages of lesion development, this possible effect may be combined with the paradoxical but general antiproliferative effects of most hepatocarcinogens (Farber et al. 1976).

Hepatocellular regeneration (HR)--Typically associated with MH, but also found independently, were discrete foci of hepatocellular regeneration, usually in hyperplastic form. The foci were of variable size and were randomly distributed. These scattered islands were composed of small, moderately basophilic hepatocytes with normal-sized or smaller than normal-sized nuclei arranged in hyperplastic (more than two cells thick) muralial cords. These foci did not contain iron pigment but did contain periodic acid-Schiff (PAS)-positive material, presumably glycogen. The islands were not well demarcated from the surrounding parenchyma except in cases where the remainder of the liver was homogeneously necrotic and/or affected with MH, and there was no visible compression of adjacent parenchyma (Fig. 6). These islands occurred in the presence of hepatocellular degenerative lesions, including frank hepatocellular necrosis and were probably a compensatory response to the degenerative effects and cytotoxic effects of potential hepatotoxins and/or hepatocarcinogen(s) or non-specific necrogenic agents.

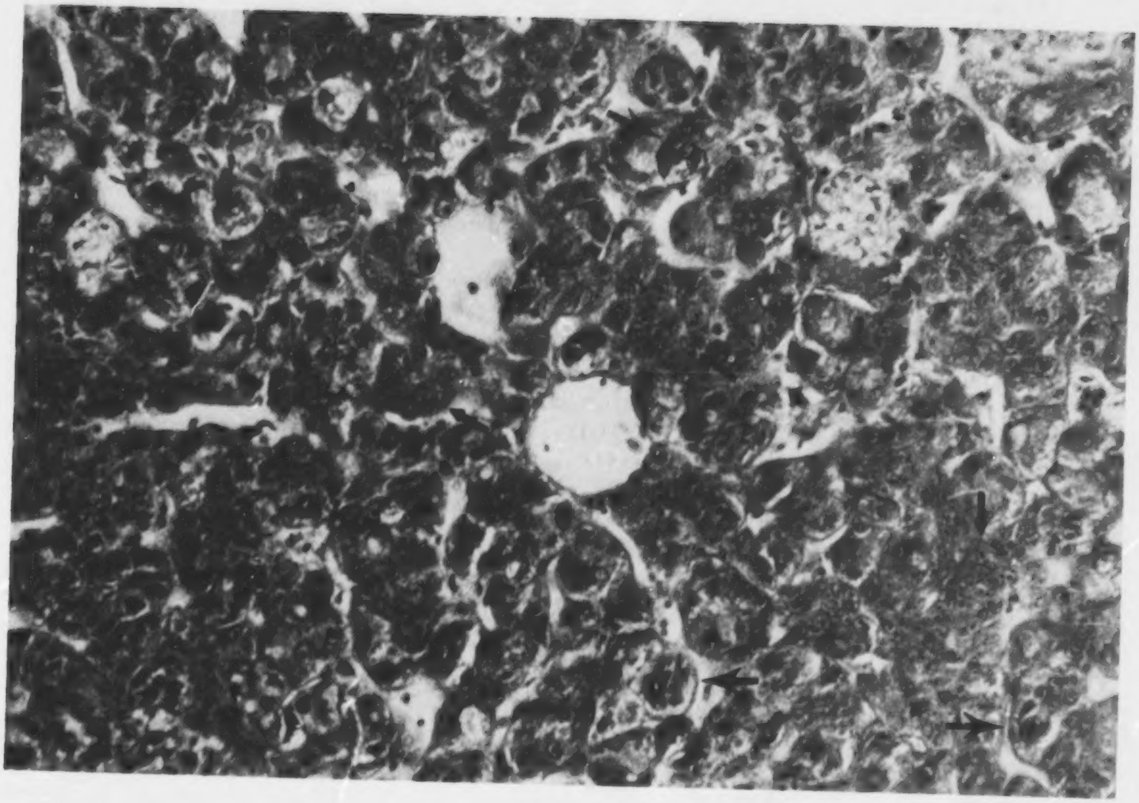

FIGURE 6. Micrograph of the hepatic lesions megalocytic hepatosis and hyperplastic hepatocellular regeneration in English sole. In this liver section, numerous megalocytic hepatocytes (megalocytic hepatosis) indicated by large arrows, were accompanied by a hyperplastic proliferation of small, basophilic, regenerative hepatocytes in a multifocal pattern (small arrows). H & E, 480X.

The proliferative capacity of cells comprising these islands suggests that they have developed a selective resistance to the necrogenic effects of putative hepatotoxin(s)/carcinogen(s), as proposed by Farber (1976) in his model of experimental rat hepatocarcinogenesis. These islands are probably metabolically immature as evidenced by their inability to take up or store iron (Williams 1976). These regenerative, undifferentiated hepatocytes may, in fact, be unable to transport and/or bioactivate hepatotoxins or hepatocarcinogens as indicated by Solt et al. (1977) and Farber (1976). Although our research has explored very few indices of maturation, this interruption of maturation is thought to be a constant characteristic of the pathway of differentiation leading to neoplasia (Farber et al. 1977, Solt et al. 1977), and is a functional manifestation of anaplasia (reversion to a more embryonic form of the cell type in question). Therefore, these islands may represent preneoplastic lesions, or at least precursors of preneoplasia, in the stepwise selection process progressing towards the autonomous growth seen in neoplasia (Pitot 1977).

On the other hand, these islands may be "remodeled" (Kitigawa 1976) back into the normal liver parenchyma by differentiating into mature hepatocytes, or they may simply regress. They are not considered to possess the property of autonomous growth (neoplasia), and therefore, are considered reversible lesions. Such "remodeled" cells, however, may later give rise to neoplasia if a proper stimulatory agent is supplied, and are considered to be "initiated" hepatocytes in the two-step initiation-promotion model of hepatic hyperplasia (Pitot 1977). In our study, hepatocellular neoplasms and foci of hepatocellular atypia were found adjacent to or within these regenerative, hyperplastic islands.

Hepatocellular eosinophilic hypertrophy (HEH)--Also commonly associated with MH, but often as an independent lesion, were discrete hepatic foci or micronodules characterized by a dense cytoplasmic eosinophilia, often with accompanying increase in cytoplasmic diameter, termed eosinophilic hypertrophy or eosinophilic foci (Fig. 7). The nuclei were vesicular, normal-appearing, or contained condensed chromatin elements. Densely eosinophilic perinuclear inclusions were often visible in the more pale eosinophilic cytoplasm. The significance of or nature of these inclusions is not presently known. The cytoplasm was rich in glycogen, with reduced basophilia and did not contain stainable iron, indicating an inability to store or take up iron. This absence of stainable iron-containing pigment parallels the findings for regenerative, hyperplastic islands. Pyknotic nuclei and cytoplasmic degeneration were rare. Occasionally, within these typically spherical, multiple micronodules were groups of hepatocytes which were eosinophilic and hyperplastic and lacked the normal muralial structure. These eosinophilic foci were often found closely opposed to or even within larger multifocal to diffuse areas of hepatocellular hyperplasia/regeneration. There was no parenchymal compression associated with these foci.

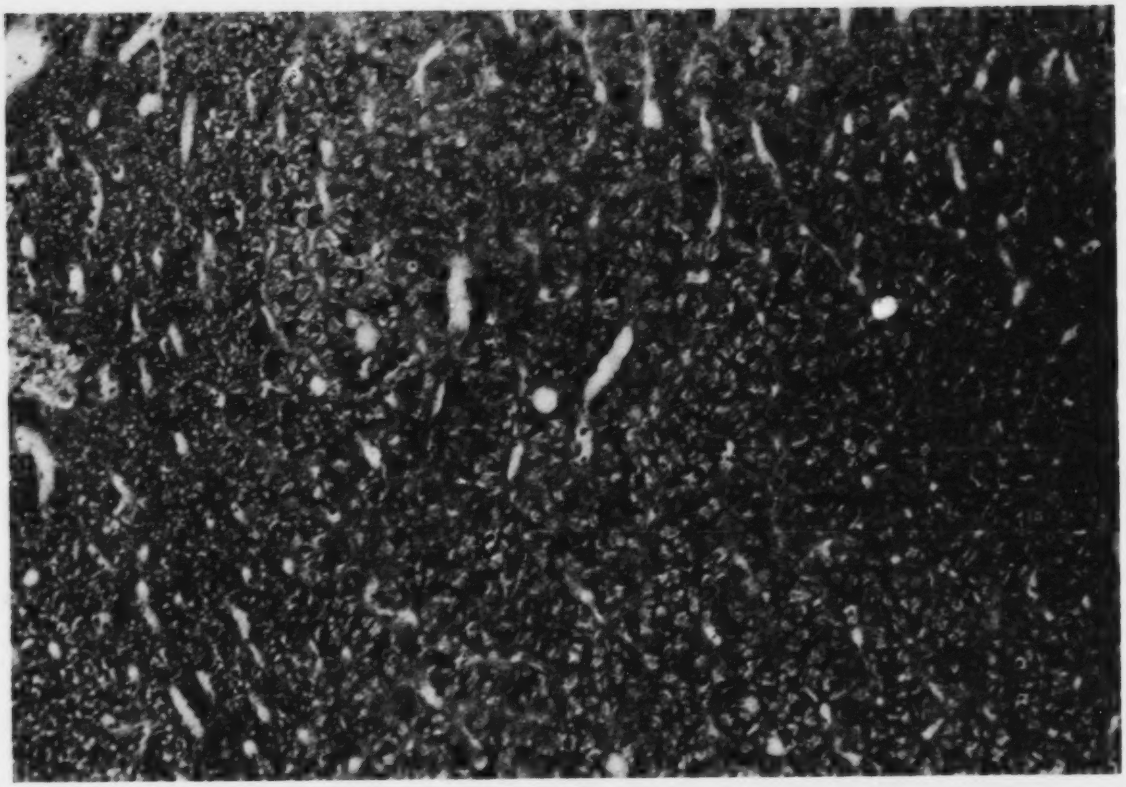

FIGURE 7. Micrograph of nodular hepatocellular eosinophilic hypertrophy in English sole. In the right hand section of this micrograph is a hepatic nodule, characterized by hypertrophied, eosinophilic hepatocytes within an organized muralium, with a low degree of cellular atypia. This lesion is diagnosed as nodular hepatocellular eosinophilic hypertrophy. H & E, 240X.

These foci are similar morphologically to the enzyme-deficient, altered foci described as preneoplastic lesions in rats (Solt et al. 1977, Scherer and Emmelot 1975, Bannasch 1976, 1978). Their rich glycogen stores suggest a retarded rate of glycogen metabolism, also seen in similar rat lesions (Solt et al. 1977, Bannasch 1976). Such foci may be precursors of neoplasia (Rabes et al. 1972), but are also considered reversible. Some authors (Rabes et al. 1972) consider these enzyme-deficient areas to be an essential but not sufficient prerequisite for microcarcinoma formation. The fact that these eosinophilic foci are found in livers with true hepatocellular neoplasms suggests a role in the progression towards neoplasia in the English sole. Similar lesions have been detected in rats exposed to the PCB Aroclor 1260 (Kimbrough et al. 1975).

Minimum deviation nodules (MDN)--Commonly found in English sole livers bearing one or all of the previously described lesions were small hepatocellular nodules. Termed minimum deviation nodules (MDN), they had several morphologic features typical of neoplasia, including cytoplasmic hyperbasophilia, changes in hepatocellular polarity, a slightly increased nuclear to cytoplasmic ratio by virtue of nuclear enlargement, prominent nucleoli, slight compression of the adjacent parenchyma, absence of other hepatic elements and a total absence of stainable cytoplasmic iron (Fig. 8). Hyperplastic hepatocytes were often located within these nodules.

These proliferative nodules are thought to arise from the regenerative, hyperplastic islands, as similar nodules emerge from areas of hyperplasia in rats (Rabes et al. 1972, Pitot 1977, Farber 1976, Solt et al. 1977, Squire and Levitt 1975, Newberne 1976, Karasaki 1976, Bannasch 1976, Weisburger et al. 1972). These cells are putatively the result of a complex sequential selection process (Scherer and Emmelot 1976) in the hepatocyte population. They most probably originate from within the previously selected hyperplastic, regenerative foci, and may be resistant to the antiproliferative, degenerative effects of hepatotoxin(s)/carcinogen(s). MDNs correspond closely with the hyperbasophilic foci (Bannasch 1976, Karasaki 1976, Rabes et al. 1972) which are considered irreversible lesions in neoplastic progression, capable of autonomous growth. This issue is controversial due to the lack of consistency in both transplantation success with this type of lesion (Ward and Vlahakis 1978) and cell culture growth characteristics (Karasaki 1976) and the inadequate predictive ability of malignancy provided by morphologic features (Newberne 1976).

Liver cell adenoma (LCA)--These supposedly benign neoplasms exhibited heterogeneous microscopic features. They were usually spherical nodules with clearly demarcated borders, and were composed of basophilic, enlarged hepatocytes with intact liver cord architecture, but occasionally showed an eosinophilic or amphophilic cytoplasm (Fig. 9). The cytoplasm of individual hepatocytes composing the nodules was sometimes heavily vacuolated but did not contain iron pigments. There was no appreciable fibrosis, but sometimes the nodules were encapsulated. Tumor growth was expansive and not overtly invasive, and the nodules showed an

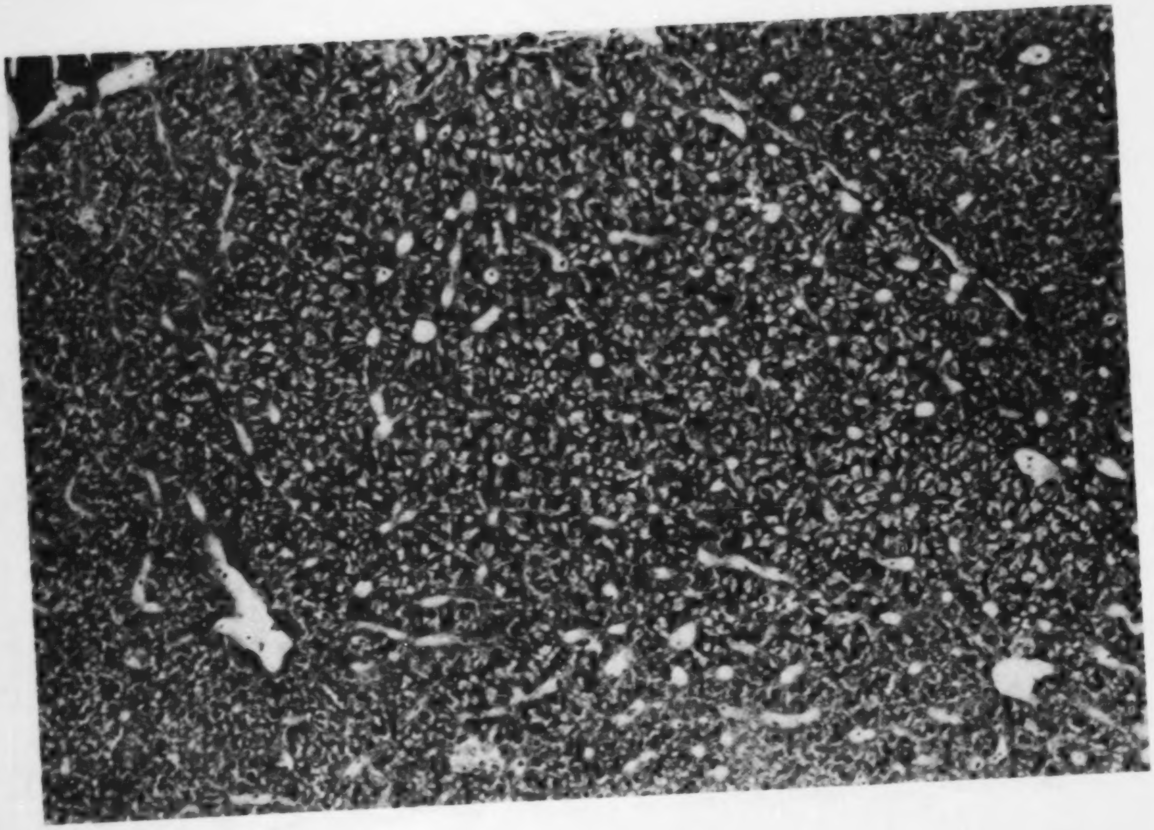

FIGURE 8. Micrograph of the hepatic neoplastic lesion termed a minimum deviation nodule in English sole. A small nodule composed of slightly hypertrophic, basophilic hepatocytes is seen at the center of the micrograph. This type of nodule is classified as a minimum deviation nodule. H & E, 240X.

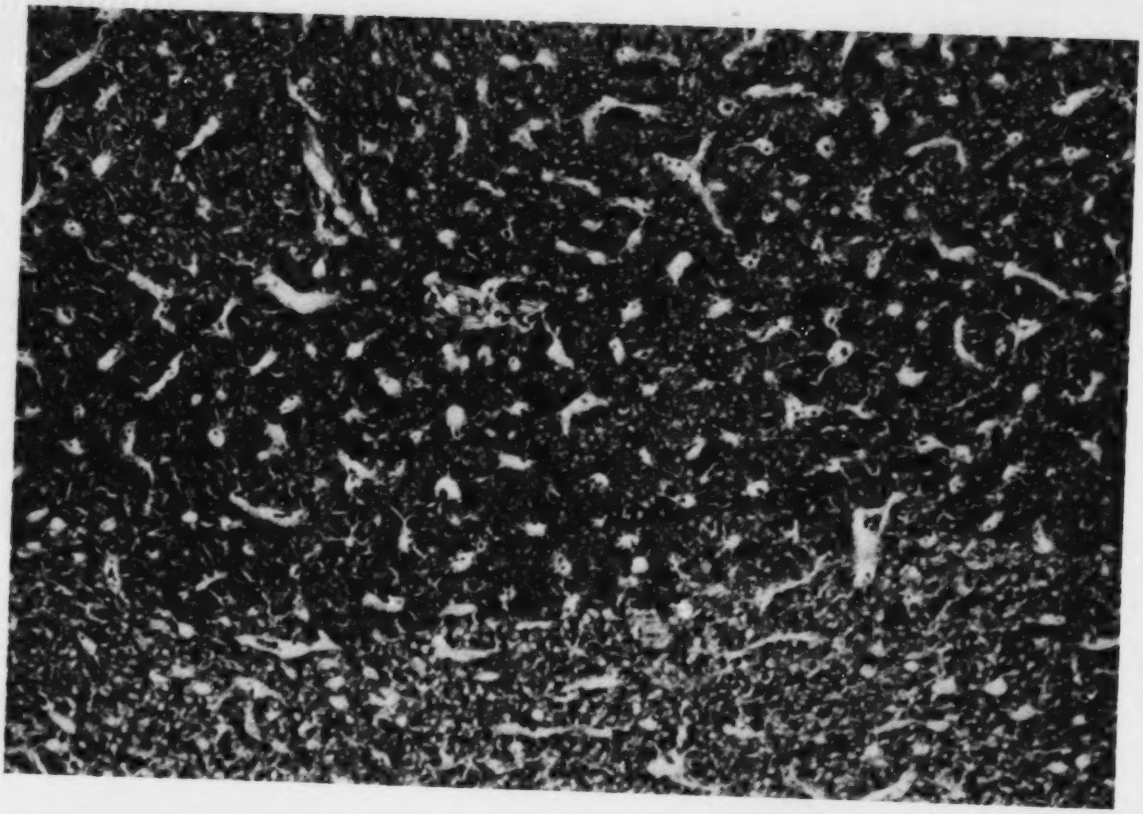

FIGURE 9. Micrograph of a liver cell adenoma in English sole. The upper portion of this micrograph contains a large hepatic nodule composed of an organized proliferation of basophilic hepatocytes, characteristic of a liver cell adenoma. H & E, 240X.

increased cellularity in comparison to the surrounding parenchyma. When stained with Masson's Trichrome (Preece 1972), these neoplasms exhibited a rich maroon-brown cytoplasmic staining. The nodules had a relatively normal-appearing muralial architecture, and lacked of bile ducts and melanomacrophage centers (MMCs) (Roberts 1975, Agius 1979). The nodules were sometimes slightly anaplastic; in which case, the hepatocytes had prominent nucleoli. Monomorphic cell populations were often observed within individual nodules, retaining polarity, as was occasional hyperplasia and thickening of hepatic cords.

Extrahepatic metastases of this type of neoplasm have never been found in English sole from Puget Sound, but multiple LCA were located in individual livers, suggesting either an independent, multifocal origin, or intrahepatic metastases. The classification of LCA was based solely on histologic criteria typical of similar non-metastasizing, benign hepatocellular neoplasms from human studies and mammalian models (Squire and Levitt 1975, Edmondson 1958, Newberne and Butler 1978).

Hepatocellular carcinoma (HC)--Other hepatocellular neoplasms with widely-accepted histologic criteria characteristic of hepatocellular carcinomas in experimental animals were also found in English sole. These tumors were typically multiple and/or adjoined, with irregular borders, and displayed foci of invasion with considerable compression in growth-by-expansion (Fig. 10). The muralial architecture of the neoplasms was typically trabecular. They were composed of anaplastic, polygonal, pleomorphic hepatocytes with an increased nuclear-to-cytoplasmic ratio, vesicular nuclei with prominent nucleoli, and loss of hepatocyte polarity in relation to the sinusoids. Extrahepatic metastases, tumor emboli, and vascular invasion were not observed. The degree of cytoplasmic basophilia in these presumptively malignant neoplasms was greater than in any of the hepatic neoplasms encountered. Glycogen levels were low, possibly due to development of a Warburg-type of glycolysis present in most hepatocellular carcinomata (Bannasch 1976). Bile ducts and MMCs were absent and cytoplasmic iron was not demonstrated in the neoplastic hepatocytes. As with all of the hepatocellular neoplasms in English sole, mitotic figures were rare. Often up to 75% of the liver at a particular cross section was replaced by the neoplasm. With Masson's trichrome staining, the hepatocellular cytoplasm exhibited a purple-green tint distinguished from the red-maroon of normal hepatocytes and the denser maroon typical of hepatocellular adenomata.

Hepatocellular carcinomas exhibited morphologic heterogeneity. Most were overtly trabecular, while some were more densely cellular and solid, growing in broad sheets with little recognizable cordal pattern. Others had a pseudotubular, pseudoacinar morphology. Varying degrees of bile duct proliferation and fibrosis were seen associated with the periphery of most of these neoplasms.

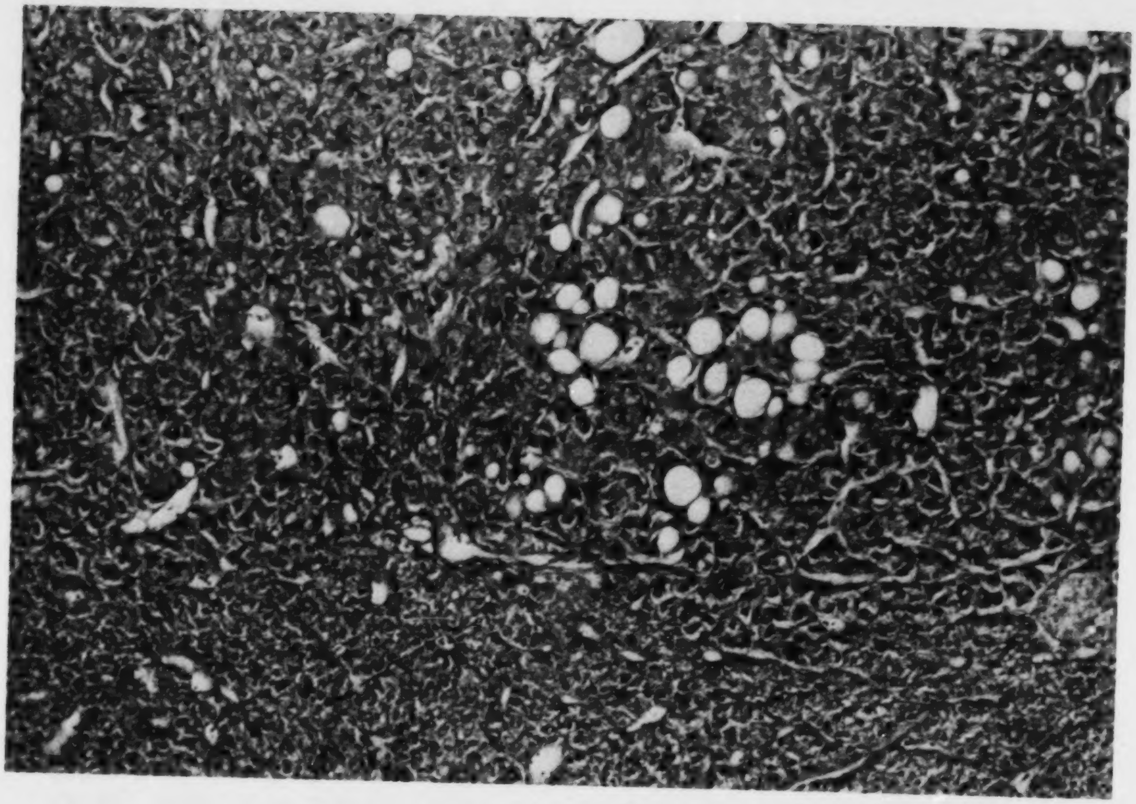

FIGURE 10. Micrograph of an hepatocellular carcinoma in English sole. The upper section of this micrograph shows an hepatic nodule with a disorganized muralial architecture, composed of pleomorphic, anaplastic hepatocytes which have lost the usual orientation (polarity) to the sinusoids. This morphology is characteristic of an hepatocellular carcinoma. H & E, 240X.

Similar neoplasms have been induced in mammalian models by a wide variety of natural and xenobiotic hepatocarcinogens (Farber 1976). These compounds include pyrrolizidize alkaloids, azo dyes, chlorinated biphenyls (Kimura and Baba 1973, Ito et al. 1973a, Kimbrough and Linder 1974, Kimbrough et al. 1975), and benzene hexachloride (Ito et al. 1973b). Hepatocellular carcinomas are also associated with high hepatic PCB levels in Atlantic hagfish (*Myxine glutinosa*) (Falkmer et al. 1977), and have been found in Atlantic tomcod (*Microgadus tomcod*) from polluted waters of the Hudson River (Smith et al. 1979).

Cholangiocellular carcinoma (CC)--A separate class of intrahepatic proliferative lesions in English sole was clearly cholangioproliferative. The most common type was a cholangiocellular carcinoma and was manifested by a disorganized proliferation of cholangiolar epithelium assuming a tubular pattern. These lesions were grossly visible as pale green nodules with highly irregular borders, and were often fungiform with numerous fingers insinuating throughout the hepatic parenchyma. Microscopically, the tubules were usually very irregular in architecture, or were simply absent, with the neoplasms consisting of a highly cellular proliferation of squamous to cuboidal biliary epithelium, usually within a fibrous stroma (Fig. 11). These tumors in English sole could be differentiated from hepatocellular neoplasms by virtue of their tubular structure and the distinct nuclear morphology of the tumor cells. Nuclei of biliary epithelium are much smaller and more ovoid than hepatocellular nuclei, and possess a less prominent nucleolus and less nuclear chromophilia. The degree of cytoplasmic basophilia in cholangiocellular neoplasms was considerably less, while the cytoplasmic eosinophilia (H & E stain) was slightly greater than the hepatocellular counterpart.

Although these neoplasms were not particularly anaplastic, they generally were extremely disorganized and tubular structures resembling mature bile ducts were infrequently observed. They were also often highly invasive, replacing large portions of the liver.

Although for most of these neoplasms, malignancy was presumed from their cytologic features, the malignancy of one individual neoplasm was clearly demonstrated in an English sole from the Duwamish River. This extremely invasive neoplasm similar in morphology to other liver neoplasms in English sole diagnosed as cholangiocellular carcinomas, almost entirely replaced the posterior hepatic lobe. The invasive capacity of this neoplasm was so great that the available blood supply was outgrown, and vascular channels were invaded, resulting in massive areas of both ischemic and hemorrhagic necrosis. The most significant findings, however, were extrahepatic metastases in the spleen, kidney, small intestine muscle wall, and ventricular myocardium. In the kidney, heart and spleen, these metastases had seeded successfully and were invading and replacing adjacent tissues. These metastatic foci were identical histologically to the massive primary carcinoma in the liver.

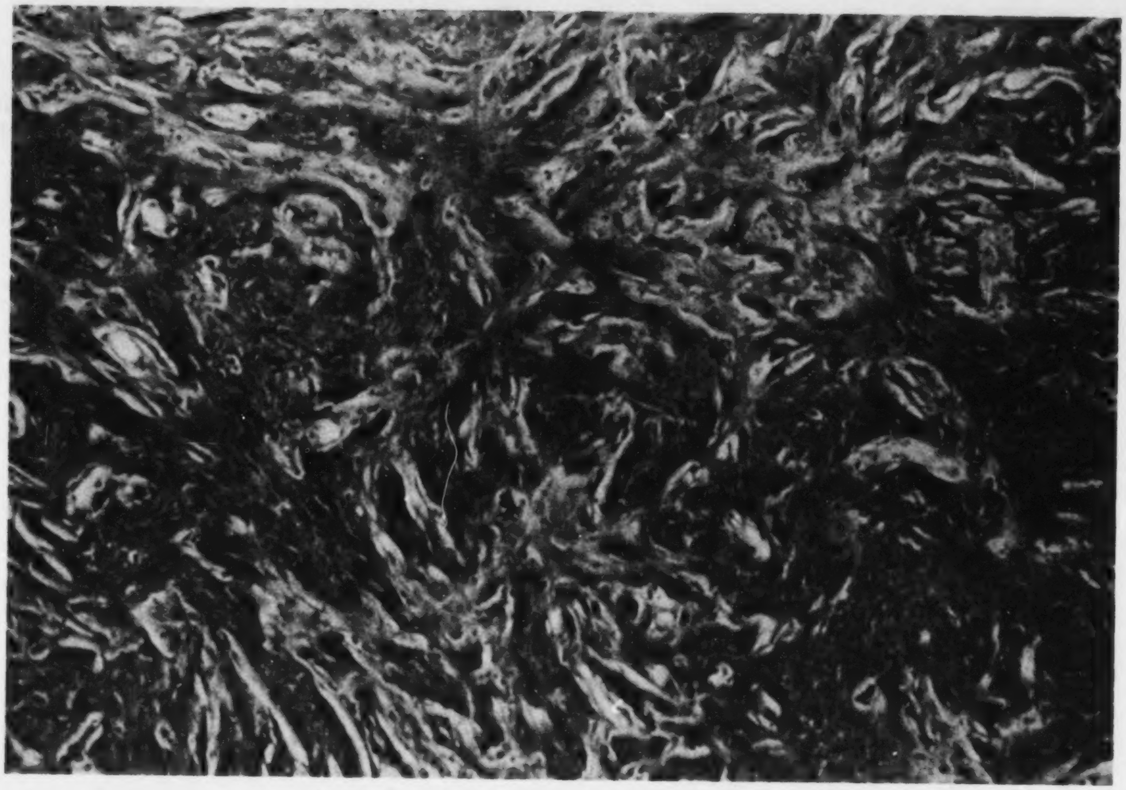

FIGURE 11. Micrograph of a metastasizing cholangiocellular carcinoma in English sole. This disorganized neoplasm in the liver was composed of pleomorphic, invasive cells closely resembling biliary epithelium and had metastasized to the heart, spleen, kidney and gut wall musculature. H & E, 240X.

The cell of origin in cholangiocellular carcinoma observed in English sole was most probably biliary epithelium from the vicinity of the ducts of Hering (Moulton 1978). However, there is controversy over this issue. Exposure to most hepatocarcinogens often produces a proliferation of bile ducts which may lead to a cholangiocellular carcinoma (Farber 1976). But with the carcinogenic azo dyes there is an induction of both cholangiocellular and hepatocellular carcinoma (Farber 1956). These carcinomas may arise from tubular stem cells which can develop into either type of malignancy. Such a mechanism may be operant in English sole since numerous examples of both separate hepatocellular and cholangiocellular carcinomas were encountered in individual livers, and mixed carcinomas were also seen. Both mature epithelial cell types do arise from the same embryonic stem cell in mammals. Similar tumors in rats and mice have been classified as tubular variants of a hepatocellular carcinoma (Squire and Levitt 1975, Jones and Butler 1975). The clear morphologic differences between cholangiocellular carcinomas and hepatocellular carcinomas described here permit such a distinction in the case of English sole. Similar neoplasms diagnosed as cholangiocellular carcinomas as well as mixed carcinomas have been reported in hagfish (Falkmer et al. 1977).

Cholangiofibrosis (CF)--A separate cholangioproliferative lesion, thought to be non-neoplastic by some (Farber 1976) and preneoplastic by others (Reuber 1968) was classified as cholangiofibrosis (CF)/adenofibrosis (Squire and Levitt 1975) in English sole. This lesion consisted of a proliferation of dilated cystic tubular structures, probably bile ducts, in a dense encapsulating and enveloping fibrous stroma, with only occasional atypia in the bilary epithelium. Compression and invasion were absent, and because of the fibrosis and delimiting encapsulation, the lesion in English sole was considered to be a benign proliferation. Reuber (1968) believes that CF precedes development of well-differentiated cholangiocarcinoma. No evidence to support this has been found in English sole. As Farber (1976) and Ward and Vlahakis (1978) suggest, most hepatocarcinogens are also inducers of proliferation of bile ducts in the liver early in carcinogenesis. With hepatocarcinogens, this proliferative response regresses or may develop into cholangiofibrosis, but it may also lead to the development of a hepatocellular carcinoma.

This lesion has been induced in rats, mice, and hamsters by xenobiotics including 2-acetamidofluorene and 2-diacetamidofluorene (Reuber 1968), and PCBs (Kimbrough et al. 1972, Kimbrough and Linder 1974).

Hemangioma--Two cases of lesions which closely resembled a mammalian hemangioma were found in the livers of Duwamish River English sole. These neoplasms were characterized by a proliferation of vascular endothelial cells surrounding blood-filled spaces, supported by a thin, fibrous stroma. Often these cystic vessels were ruptured, with necrosis in the hepatocellular parenchyma. There was no invasion of peripheral tissues and the neoplastic cells showed no cellular atypia. Similar benign, hemangiomatous neoplasms have been induced by urethane injection (Heston et al. 1960, Kawamato et al. 1961, Roe 1954) and dimethylnitrosamine (Jones and Butler 1975) in experimental mammals.

Hepatocellular necrosis (HN)--Other incidental hepatic lesions were found in English sole. The most common was hepatocellular necrosis (HN)/ degeneration (not associated with incidental parasitism), a non-specific response to toxins or pathogens.

In some livers, necrosis was diffuse, affecting 75-80% of the liver, with only a minimal cellular inflammatory response. If a biologic agent were present, the cellular inflammatory response should have been prominent. Most degenerative/necrotic foci were found in conjunction with previously described lesions, and were associated with microinvasion by neoplasms, or were equated with the degenerative changes found in MH. In cases where benign neoplasms were present, or where HN was the only visible lesion, the necrotic foci may have represented necrogenic action by toxic chemicals or carcinogen(s).

Miscellaneous non-parasitic liver lesions--Fatty change (FC), or abnormal accumulation of lipid in groups of hepatocytes was also frequently encountered, both in fish with and without neoplasms. This lesion is a degenerative lesion, commonly associated with dietary deficiencies, or toxic chemical administration. Its role, if any, in the progression of lesions towards tumor formation in the English sole is not presently known.

Many English sole livers from the Duwamish Waterway exhibited elevated levels of intracellular iron when stained by Prussian Blue reaction. (This reaction is mainly exhibited by hemosiderin.) This accumulation is especially common in the megalocytic cells of MH. Iron accumulation can be associated with non-specific liver injury (Robbins and Cotran 1979) but is also associated with a wide spectrum of factors, including excessive dietary iron, anemia, liver cirrhosis, and hemorrhage. The identification of this condition, termed hemosiderosis (Hem), generally indicates the existence of some concurrent condition responsible for the excess iron (Robbins and Cotran 1979).

The final hepatic lesion to be described is rarely found in the English sole, but is more common in the starry flounder. A detailed description will be presented in the section leading with hepatic lesions in starry flounder. This lesion most closely resembles the human hepatic condition peliosis hepatis (PH) and is basically characterized by severe congestion/ectasia of the sinusoids with intra-sinusoidal fibrin deposition (resembling cysts containing blood) hemorrhage, and necrosis of the hepatic cords adjacent to the foci of ectasia (dilatation).

Parasitic hepatic lesions--The most frequent and widely distributed parasitism of hepatic tissue was an infection by members of the sporozoan genus Myxidium (Butschli), family Myxidiidae (Thelohan). The infection was generally confined to the intrahepatic bile ducts and bile ductules (cholangioles) where plasmodial forms of this cnidosporidan were present. Spores were also present adjacent to the mucosa of the gallbladder. Commonly, the plasmodia were of insufficient volume to cause compression atrophy of the biliary epithelium, and the typical host response was a mild peribiliary lymphoid infiltrate. In more severe cases, there was a proliferation of bile ductules containing plasmodia and spores, and variable degrees of fibrosis with chronic inflammation. In the most severe cases, where the parasite could be found scattered throughout the hepatic parenchyma in addition to the intrahepatic bile ducts and bile ductules, severe fibrosis and fibroplasia were present along with hepatocellular necrosis affecting a significant proportion of the liver. Varying degrees of cholestasis (bile stasis) were also observed in severe cases. These severe infections, with significant hepatic fibrosis, were interpreted as true pathologic lesions due to the amount of parenchyma replaced by fibrous tissue. However, the functional effect of these lesions is not known.

Prevalence of liver lesions--

The livers of English sole from McAllister Creek and the Snohomish River sampling sites (a total sample of 73 fish) exhibited only low prevalences of hemosiderosis, fatty change, and non-specific hepatocellular necrosis, and contained none of the other degenerative, potential preneoplastic, and true neoplastic lesions found in both the Duwamish Waterway and Lake Washington Ship Canal (Fig. 12). The prevalences of all three of these lesion categories were significantly higher ($p<0.001$) in the Duwamish Waterway than in any of the other areas. Twelve of the 16 major types of hepatic lesions were found only in English sole from the Duwamish Waterway and the Lake Washington Ship Canal (551 and 49 livers, respectively, were examined). These lesions included MH, HNH, HR, CC, MC, MDN, LCA, HC, CF, PH, HEH and hemangiomas. The prevalences of fish with neoplastic lesions ranged from 2.0% (CC) to 7.1% (LCA) in Duwamish River English sole (overall prevalence) while in the Lake Washington Ship Canal the average prevalence of fish with hepatic neoplasms ranged from 0% (CC) to 4.1% (HC). For fish with non-neoplastic hepatic lesions, prevalences ranged from 0.5% (3 of 551 fish) for peliosis hepatis to 18.5% (101 of 551 fish) for megalocytic hepatosis in the Duwamish Waterway and in the Lake Washington Ship Canal from 0 (PH) to 12.2% (MH).

Within the Duwamish River estuary, only Stations D and G had idiopathic hepatic lesion frequencies significantly higher ($p<0.05$) than those found at the other Stations (Fig. 13). The overall prevalence of HN and CC was highest at Station D, and the prevalence of HNH and Hem was highest at Station G. In general, the prevalence of sole with liver lesions tended to be lower at Stations A and B as compared to the other sampling stations. This observation is suggested by the fact that the prevalence of only 2 to 3 of the 13 lesions shown in Figure 13 were higher than 5% in sole from Stations A and B, while prevalences of between 6 and 11 of the 13 lesions were higher than 5% in sole from the other stations. In view of this general trend, it is surprising that the highest prevalence of MH was found at Station A.

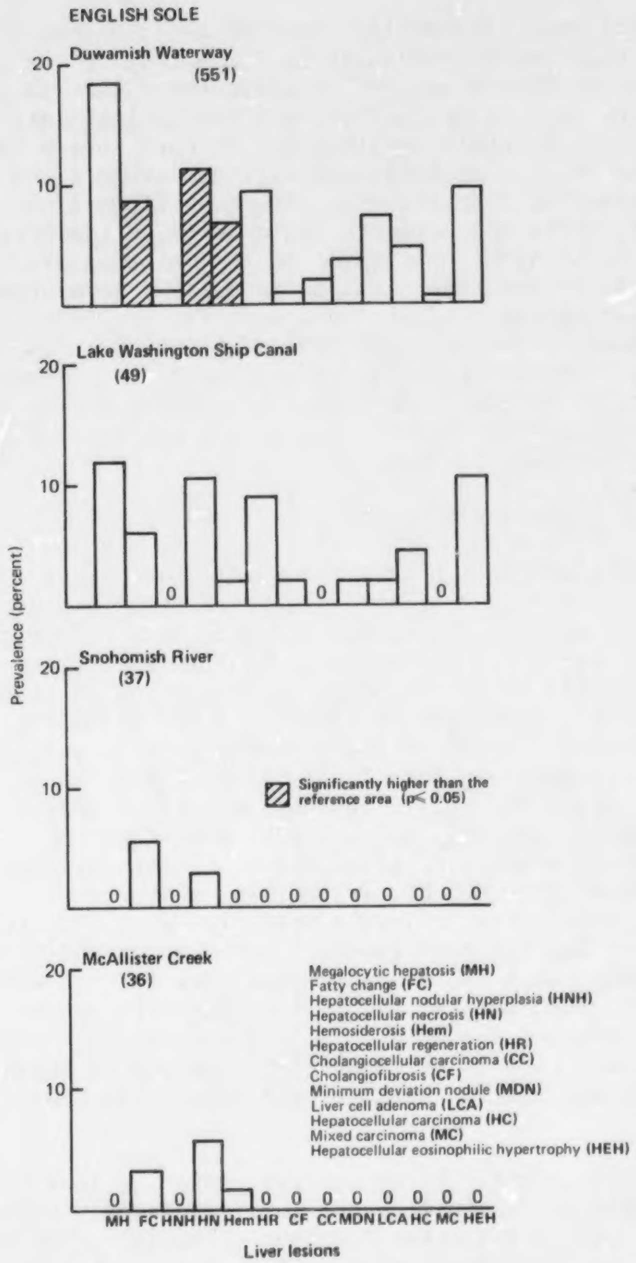

FIGURE 12. Prevalences of idiopathic liver lesions in English sole from the Duwamish Waterway, Lake Washington Ship Canal, Snohomish River, and McAllister Creek sampling areas.

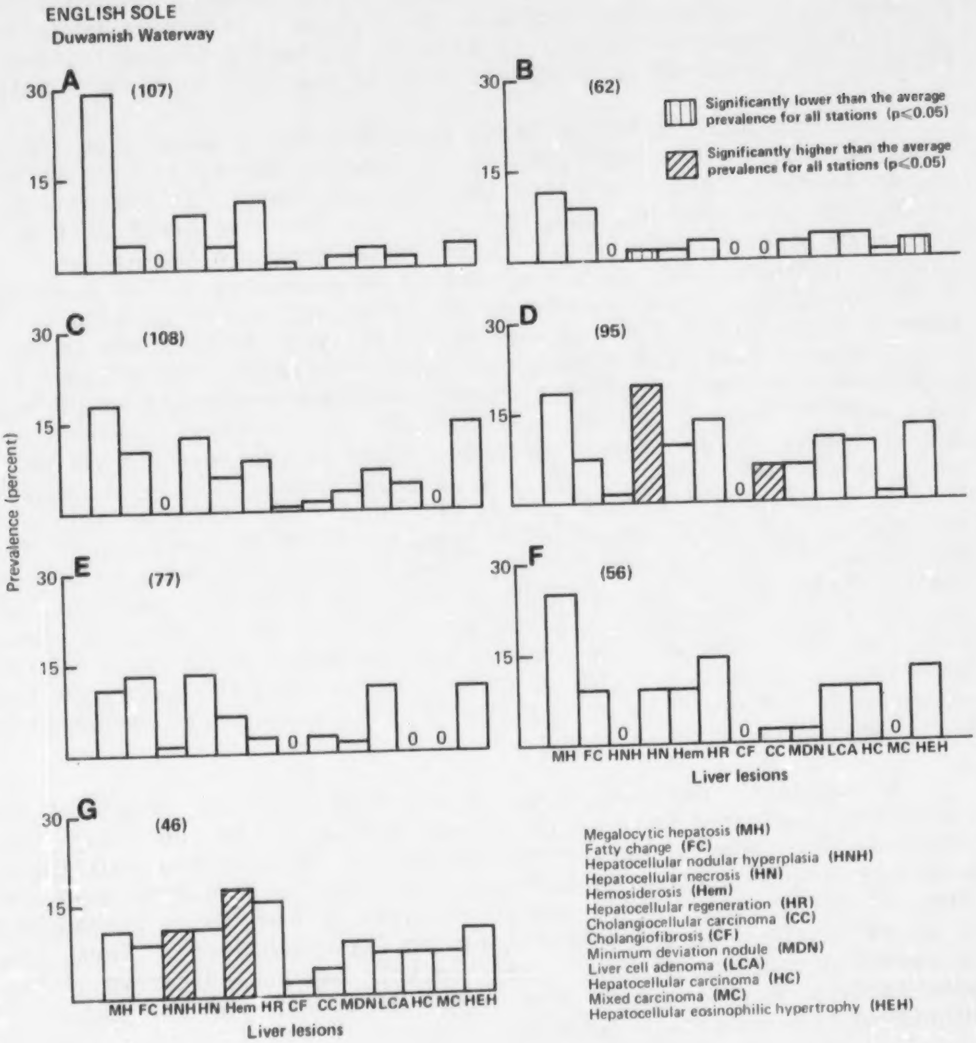

FIGURE 13. Prevalences (by station) of idiopathic liver lesions in English sole from the Duwamish Waterway.

Prevalences of hepatic neoplasms and "preneoplastic" lesions (HR and HEH) are presented in Figure 14. The overall prevalences of sole with one or more of these types of lesions were essentially the same in the Duwamish Waterway (20.%) and the Lake Washington Ship Canal (20.4%). The overall prevalences of sole with liver neoplasms alone from the Duwamish Waterway and Lake Washington Ship Canal were 12.9% and 8.2%, respectively. The close association between the suspected preneoplastic lesions and neoplasms in English sole livers is reflected by the much higher prevalence of fish having both types of lesions, 17.2% in the Duwamish Waterway and 8.4% in the Lake Washington Ship Canal.

When the hepatic lesion prevalence data for English sole from the Duwamish Waterway are analyzed from a temporal approach, some striking trends emerge. Table 4 depicts the temporal changes in the prevalence of English sole with MH, HEH, MDN, LCA, and HN. The great differences that were observed between the lesion prevalences in two winter seasons (winter 1979 and 1980) do not appear to be accounted for by seasonal variation alone. However, due to the relatively short duration of this survey, the possibility of a periodic multi-year fluctuation in the prevalence of these conditions cannot be eliminated. As one means of determining if the prevalences of the lesions represented in Table 4 were significantly higher during the last two samplings (summer 1979 and winter 1980) than the prevalences found in the previous three samplings, statistical comparisons were performed. The mean prevalence of each lesion for the first three samplings (fall 1978, winter 1979 and spring 1979) was statistically compared with the prevalences for the summer 1979 and winter 1980 samplings. The prevalence of MH and HN was significantly higher ($p \leq 0.05$) in both the last two samplings, while the prevalence of HEH was significantly higher only during the winter 1980 sampling. These lesions which had increased prevalences during the last two samplings were non-neoplastic lesions thought to be acute or subacute responses by animals to relatively short-term exposure to toxic chemicals.

In contrast, the prevalences of all types of liver neoplasms except one (MDN) fluctuated only slightly over the length of the study (Table 4). MDN were significantly more prevalent during the last two samplings. Neoplasms are thought to be a chronic response to long-term exposure to chemical carcinogens, while MDN are thought by some to be preneoplastic or an early stage of a neoplastic lesion (Bannasch 1976). Thus, the increased prevalence of MDN in English sole may be indicative of the same factors responsible for the higher prevalences of the above-mentioned non-neoplastic lesions.

Interrelationships among idiopathic liver lesions--The chi-square contingency analysis was used to test the independence of the occurrence of 16 idiopathic liver lesions in Duwamish River English sole. Pathologic conditions that were found to be not independent may have a temporal and/or etiological relationship since lesions involved in a progressive sequence or induced by the same agent(s) are more likely

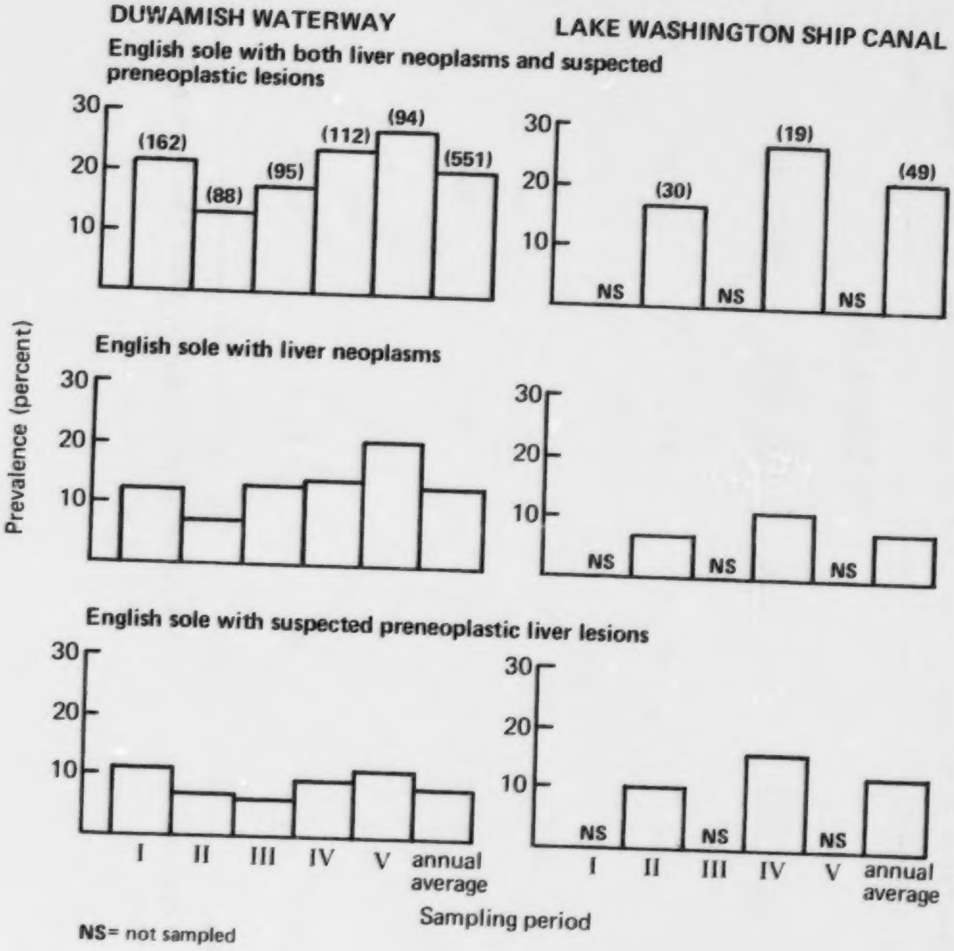

FIGURE 14. Prevalences of English sole with suspected "preneoplastic" liver lesions, liver neoplasms, and combined preneoplastic/neoplastic liver lesions from the Duwamish Waterway and Lake Washington Ship Canal.

TABLE 4. Temporal variation in the prevalence of English sole with selected liver lesions in the Duwamish Waterway. (See Table 1 for the number of fish examined during each sampling period).

Liver Lesion	Prevalence (%)					
	Oct. 1978	Jan. 1979	Apr. 1979	July 1979	Jan. 1980	Mean
Megalocytic hepatosis (MH)	14.8	14.8	14.7	22.3*	24.3*	18.5
Hepatocellular eosinophilic hypertrophy (HEH)	9.3	6.8	6.3	10.7	14.8*	9.8
Liver cell adenoma (LCA)	7.4	3.4	8.4	4.5	11.7	7.1
Minimum deviation nodule (MDN)	0.1	0	3.2	7.1*	8.5*	3.6
Hepatocellular necrosis (HN)	7.4	3.4	11.6	17.9*	17.0*	11.3

* Signifies that the prevalence was significantly higher ($p<0.05$) than the mean prevalence for the first three sampling periods.

to be observed concurrently within an animal. Nevertheless, in a study such as this one, disease etiology and the process of progression towards neoplasia can only be inferred by statistical analysis. Controlled laboratory exposure experiments are essential for establishing such relationships.

The results of the chi-square contingency tests are listed in Table 5. This table also contains values for Pearson's coefficient of contingency (PC), a measure of the degree of association between two variables (Ostle 1972). The strongest associations were generally found between the separate categories of neoplastic conditions. For example, a fish liver with a HC was very likely to possess a CC (HCxCC, PC=.33, p<0.001), or a mixed carcinoma where the neoplastic elements were not separable into distinct tumors (HCxMC, PC=.2, p<0.005). The neoplasms judged to be malignant by histologic criteria were also highly associated with their benign counterparts, (HCxLCA, PC=.27, p<0.001; CCxLCA PC=.16, p<0.05).

Hepatocellular lesions which have been suspected to be preneoplastic (HEH) were in fact highly associated with hepatocellular and non-hepatocellular neoplasms, (HEH vs. LCA, PC=.31, p<0.001; HEH vs. HC, PC=.22, p<0.005; HEH vs. MC, PC=0.19, p<0.01; HEH vs. hemangioma, PC=.16, p<0.025). HEH was also associated with a potential progenitor lesion, fatty change or clear cell foci (Jones and Butler 1975, Squire and Levitt 1975; HEH vs. FC, PC=.25, p<0.001). Another potential preneoplastic hepatocellular lesion, HNH, was found to occur in statistically significant association with a neoplasm, the mixed carcinoma (HNH vs. MC, PC=.14, p<0.05).

Among the degenerative lesions, several showed high associations with other hepatic lesions. In MH, widespread degeneration of the hepatocellular parenchyma was usually accompanied by compensatory HR (MH vs. HR PC=.27, p<0.001). This relationship was the single significant one for megalocytic hepatosis. In addition to MH, HR occurred with parenchymal fibrosis (HR vs. fibrosis, PC=.23, p<0.001) and with hemangiomatous lesions (HR vs. hemangioma, PC=.16, p<0.025).

Table 5 presents the entire matrix of liver lesions which were compared by the chi-square test of contingency. Also of significance in these comparisons are lesions which show the least association, implying independent etiologies or events that are not closely related within a certain time frame. Examples of lesions which show low association and independence are degenerative conditions compared with "preneoplastic" lesions (HN vs. HEH) and the specific degenerative condition megalocytic hepatosis (MH) compared with the supposed chronic lesion adenofibrosis/ chlolangiofibrosis (AF/CF).

Analysis of idiopathic hepatic lesions in Duwamish River English sole with respect to sex--Of the total number of English sole captured in the Duwamish Waterway, which were examined histologically, and for whom the sex was determinable (421 in the total sample), 198 or 47%

TABLE 5. Matrix of idiopathic liver lesions which were compared in order to determine the degree of association of lesions by chi-square contingency analysis in individual English sole from the Duwamish Waterway system. Values shown indicate Pearsons coefficient of contingency (PC) and the level of statistical significance (p value) respectively.

Lesion Type	MH	HR	HEH	HN	FC	Hem	HNH	LCA	HC	AF/CF	CC
	HR (PC).27 p<001*	MH .27 .001*	LCA .31 .001*	Hemangioma .15 .05*	HEH .25 .001*	FC .16 .05*	MC .14 .05*	HEH .31 .001*	CC .33 .001*	Hemangioma .33 .001*	HC .33 .001*
	CC .11 .25	Fibrosis .23 .001*	FC .25 .001*	AF/CF .13 .10	Hem .16 .05*	HC .11 .25	MDN .12 .10	HC .27 .001*	LCA .27 .001*	Fibrosis .23 .001*	Fibrosis .19 .01*
	Hem .10 .25	Hem .16 .025*	AF/CF .23 .001*	Fibrosis .11 .25	Fibrosis .13 .10	MC .09 .25	FC .10 .25	CC .16 .05*	HEH .22 .005*	HEH .23 .001*	LCA .16 .05*
	LCA .10 .25	MC .10 .25	HC .22 .005*	LCA .05 .5	LCA .12 .10	MDN .09 .25	MH .08 .25	PH .15 .05*	MC .20 .005*	MC .20 .005*	PH .15 .05*
	HNH .08 .25	HEM .09 .25	PH .20 .005	HC .05 .5	HNH .10 .25	LCA .08 .25	CC .07 .5	FC .12 .10	PH .19 .01*	HC .15 .05*	MDN .14 .1
	Hem .06 .5	AF/CF .08 .5	MC .19 .01*	HNH .05 .5	HC .10 .25	Hemangioma .08 .5	HEH .06 .5	Fibrosis .11 .25	AF/CF .15 .05*	HN .13 .10	MH .11 .25
	HC .05 .5	PH .07 .5	Hemangioma .16 p<.025*	MC .04 .75	AF/CF .09 .25	HEH .08 .5	HR .05 .5	AF/CF .11 .25	MDN .11 .25	LCA .11 .25	MC .10 .25
	PH .04 .75	HC .06 .5	Fibrosis .10 .25	HR .04 .75	MC .06 .5	Fibrosis .07 .5	Fibrosis .05 .5	MH .10 .25	Hemangioma .11 .25	FC .09 .25	HNH .07 .5
	MDN .04 .75	HNH .05 .5	HR .09 .25	MH .03 .75	Hemangioma .06 .5	CC .06 .5	HN .05 .5	Hem .08 .25	Hem .11 .25	HR .08 .5	Hem .06 .5
	HN .03 <.75	LCA .04 .75	Hem .08 .5	CC .03 .75	CC .03 .75	MH .06 .5	LCA .04 .75	MC .08 .25	FC .10 .25	MDN .05 .5	HEH .05 .5

* Signifies a significant level of association.

were males, and 223 or 53% were females. In order to determine if any hepatic lesion or lesion category was found disproportionately in fish of either sex, the percentage of male and female English sole affected with a particular hepatic lesion was computed for each lesion category (combined hepatic lesions, MH, HEH, etc.), and this value was compared to the percentage of males or females found in the general sample. Testing the null hypothesis that all hepatic lesions are unrelated to sex, the G-statistic was computed for each lesion category. Even though the percentage of males and females affected by certain lesions deviated from the sex ratio seen in the general sample, the null hypothesis could not be rejected in any instance. Therefore, these deviations from the percentages seen in the general sample are not statistically significant, and it can be concluded that neither sex is affected disproportionately by any of the hepatic lesions analyzed.

Length-age regressions in Duwamish River English sole with and without idiopathic liver lesions--Testing the hypothesis that hepatic disease in English sole may affect the length at a particular age, and therefore growth, length-age regressions were developed for (1) Duwamish English sole without hepatic lesions, (2) Duwamish English sole with hepatic lesions (including all non-parasitic hepatic lesions), and (3) Duwamish English sole with specific hepatic lesions. For each group, regression curves were fitted for males, females, and for the entire sample composing each group. A graphical representation of one of these power curve regressions is presented in Figure 15.

Comparing Duwamish English sole without hepatic lesions to Duwamish English sole with non-parasitic hepatic lesions (Fig. 15), it is graphically evident that when both sexes are combined, there is a slight reduction in length-at-age for fish affected with liver lesions. This reduction in length is 2-3% for all ages. However, when the two samples are compared using the paired sample t-test (comparing the mean length-at-age for each age class in both samples), there was statistically no significant difference between the two samples.

Considering the series of length-age plots for English sole with specific hepatic lesions, there was no clear pattern of length reduction at all ages. Fish with HN, Hem, FC, MH, HEH, LCA, CF, CC, and the grouped "preneoplastic"/neoplastic lesions, graphically showed reduced length in older fish, generally beyond age 4 or 5. This reduction approached 16% in some lesions at the 10-year age level (as for HEH). Only the regression curves for MH and CC showed reduction in length for all ages. However, in comparing mean length-at-age for normal Duwamish River English sole against mean-length-at-age for English sole with specific hepatic lesions (at all overlapping ages) by the paired sample t-test, no significant difference was observed in any of the affected groups tested. Therefore, although the regression curves suggested a reduction in length at a particular age for English sole affected with idiopathic liver lesions, this apparent trend could not be affirmed statistically over the entire age range in which English sole were affected.

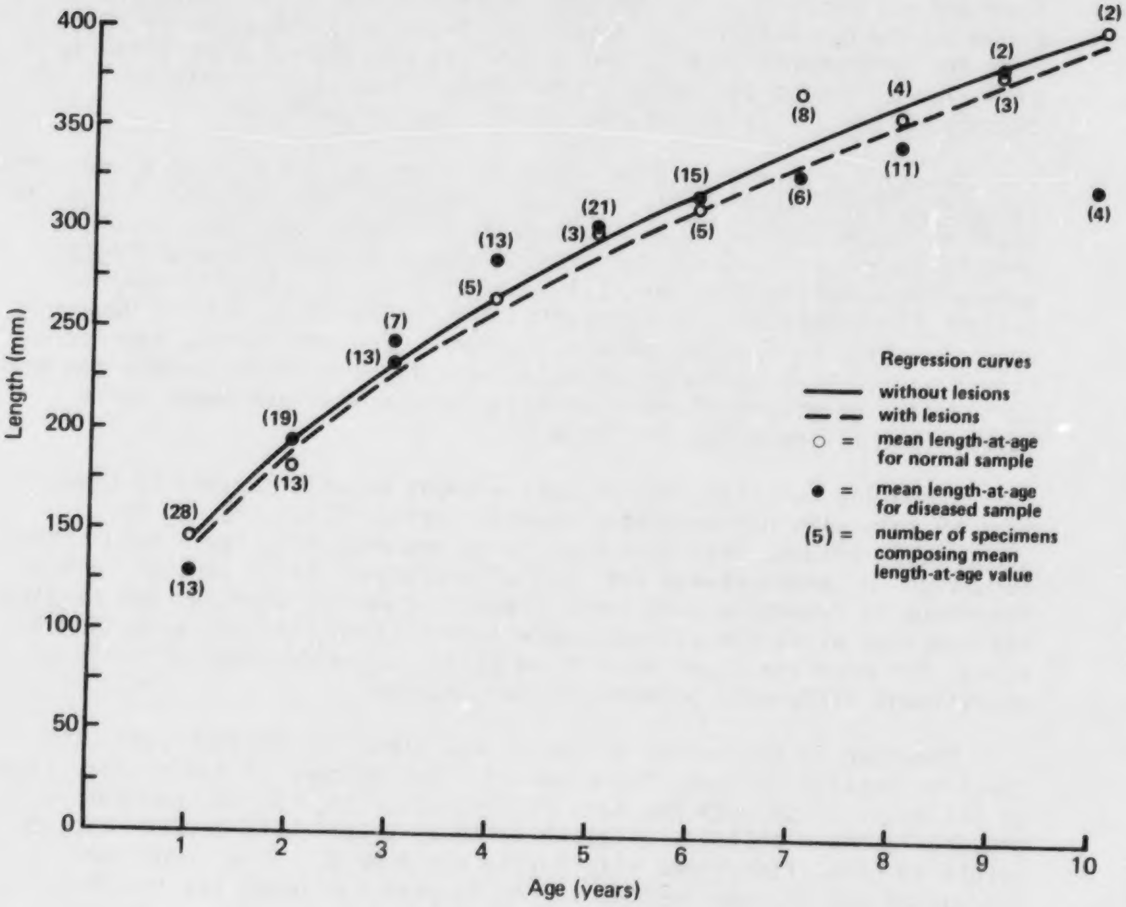

FIGURE 15. Length-age regression curves and mean length-at-age values for Duwamish River English sole with and without idiopathic hepatic lesions (both sexes combined).

Prevalence of hepatic lesions related to length (and indirectly to age) in English sole and effect of hepatic lesion presence on the length-weight relationship in Duwamish River English sole--Although the documentation of age data for our entire sample is not yet available, the parameter of length is strongly related to age, and therefore hepatic lesion prevalences in English sole were examined in three separate size groups, ≤200 mm, 201-300 mm, and ≥301 mm. From the age data presently available, English sole from the first size group (≤200 mm) are generally 1-3 years of age; English sole from the second group (201-300 mm) are 2-6 years of age; and English sole from the third group (≥301 mm) are 4-13 years of age. The fact that idiopathic hepatic lesions are clearly age-related is demonstrated in the hepatic lesion prevalence for these size groups. The first group (≤200 mm) show a prevalence of 40/279 or 14.3% (affected animal prevalence), the second (201-300 mm), a hepatic lesion prevalence of 55.3% (83 of 150), and the third size group (≥301 mm), a prevalence of 67.3%.

To investigate the possible affect of these hepatic lesions on the general health of English sole, simple length vs. weight regression curves (based on the power function $y=ax^b$ where y=weight and x=length) were fitted for Duwamish English sole which were affected with idiopathic liver lesions and for those that were not affected with idiopathic liver lesions. Similar curves were fitted (again, by the least-squares method) for English sole from the Lake Washington Ship Canal, Snohomish River and McAllister Creek sampling sites. The goal of this analysis was to find a type of "emaciation index" for each group of fish to determine if fish affected with liver lesions tended to have a reduced weight at a particular length.

The sample of normal fish was composed mainly of smaller fish (≤200 mm) and, thus, the length-weight regression was skewed because of the disproportionate number of small normal fish compared to the sample of English sole affected with liver lesions. Since this effect tends to represent older, longer fish, separate length-weight regressions were performed for the three separate length groups described previously.

In the first length group the relationship between length and weight in normal fish and those with idiopathic liver lesions was obscure. The curve for the normal Duwamish sample was almost linear up to 130 mm, indicating that normal sole had a higher weight than diseased Duwamish English sole at corresponding lengths. For fish longer than 130 mm, this relationship was reversed; diseased fish had higher weights than their normal counterparts. Diseased Duwamish English sole and normal Lake Washington Ship Canal English sole showed almost identical curves, and there were the anomalous results of lower weights for normal Snohomish and McAllister Creek English sole as compared to their diseased Duwamish English sole counterparts. Because of the conflicting, shifting, and anomalous patterns which emerged from these curves, there is probably no effect upon weight in fish within this size range due to liver lesion presence.

In the second length group, again there was no clear distinction between groups of normal fish from different sampling areas (except that Snohomish normal group showed a reduced weight compared to all other normal and diseased groups), nor was there any graphic difference between normal and diseased Duwamish English sole.

The third length group represented the older fish sampled, and therefore included a larger sample of English sole with liver lesions (74 as opposed to 36 normal Duwamish English sole). Although the differences in weight at a particular length between different curves were not statistically significant, the weight of Duwamish English sole with liver lesions at any length tended to be lower than any of the other normal groups as determined by this simple regression (Fig. 16). This suggests that liver lesions in English sole adversely affect either food conversion efficiency, the ability to compete for food, or general nutritional status, especially in older English sole. It should be reemphasized here that this apparent reduction in weight-at-length is only a trend visible in the larger length group and that these weight differences may not be statistically significant. In fact, when the data for English sole from the Duwamish River with idiopathic liver lesions is compared to similar data for English sole without these liver lesions, using the paired sample t-test comparing weight-at-length between the two samples, no statistically significant differences were found.

Characteristics of gill lesions--

Several morphologically separable gill lesions of an idiopathic, non-parasitic nature, as well as parasitic infestations were found in English sole.

Respiratory epithelial hyperplasia (REH)--The most common gill lesion, REH, consisted of a clearly hyperplastic or thickened respiratory epithelium of the gill lamellae, especially in the distal regions of the lamellae. In sole with REH, the superficial respiratory epithelium was markedly thickened, often with accompanying lamellar fusion, hyperplasia of the filament epithelium, and edema in both the lamellae and gill filaments (Fig. 17). The lesion was generally multifocal to diffuse. In this condition, respiratory exchange may be compromised due to the impaired capacity for gaseous exchange through the thickened epithelium.

Lymphocytic infiltration--A second idiopathic lesion was characterized by multifocal sites of lymphocytic and histiocytic infiltration in the subepithelial regions of the gill lamellae and filaments. There was generally no accompanying necrosis, and this condition can be interpreted as a non-specific chronic inflammatory response to injury. Response to parasites, which were commonly observed in English sole gill tissue, cannot be ruled out.

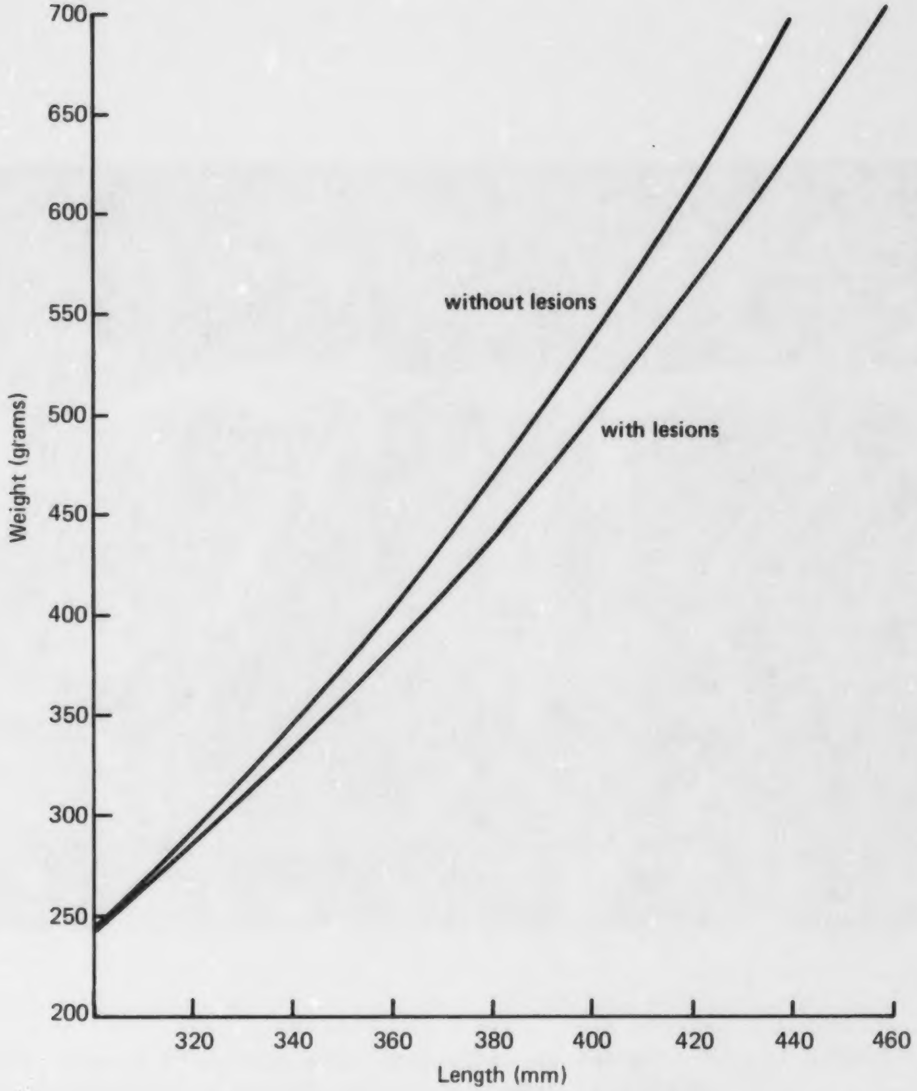

FIGURE 16. Length-weight regression curves for Duwamish River English sole with and without idiopathic hepatic lesions (least squares method).

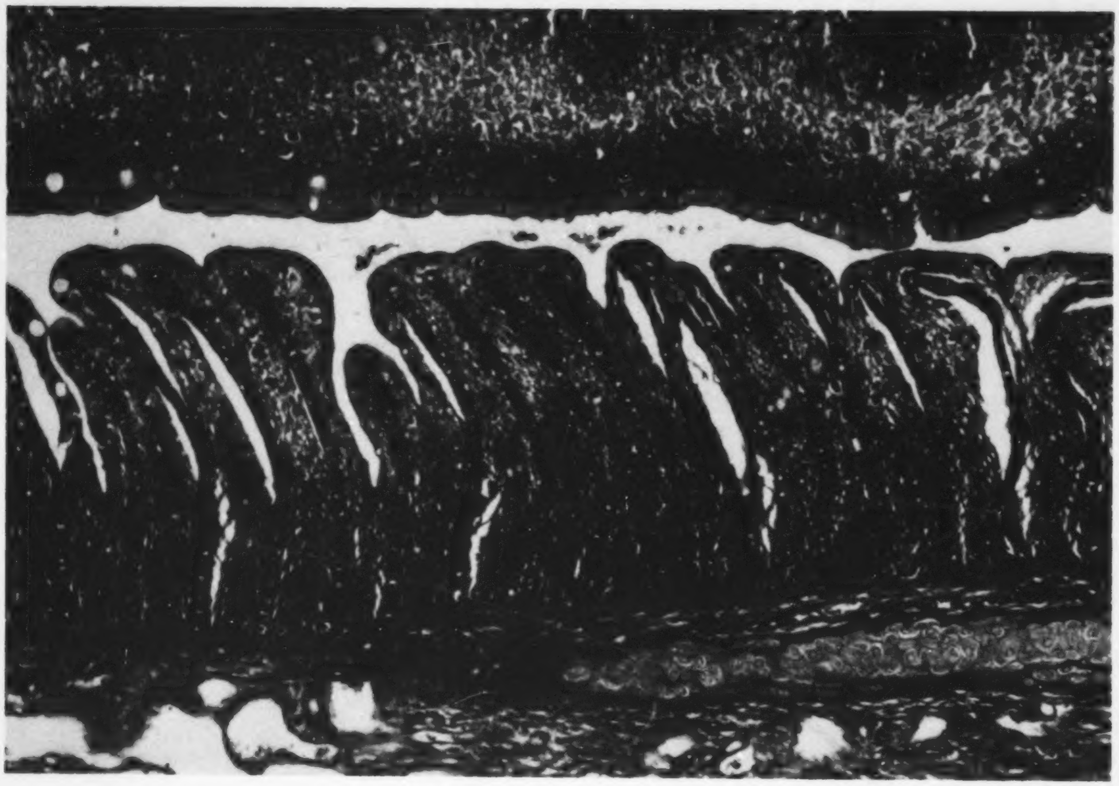

FIGURE 17. Micrograph of an English sole gill with severe respiratory epithelial hyperplasia with fusion of adjacent lamellae. H & E, 240X.

Microaneurysm--A third common idiopathic gill abnormality was represented by multiple sites of intralamellar capillary dilations, microaneurysms or telangiectasis. These occurred in absence of hemorrhage, but these dilated capillaries often contained fibrin accumulations indicative of a coagulation disorder or a tendency towards thrombosis, possibly from endothelial injury. Most commonly, though, the lesion consisted of simple capillary dilation and congestion, possibly associated with the shock response induced in capture.

Miscellaneous gill lesions--Other idiopathic lesions found at low levels only in the urban-associated estuaries were: (1) mucous cell hyperplasia (an increase in the density of goblet cells in the superficial surfaces of the lamellae and filaments); (2) lamellar necrosis (a non-specific degeneration and necrosis of the lamellar epithelium, and the capillary endothelium supporting the pillar cells and the basement membrane); (3) mixed capillary dilation and congestion (a form of lamellar microaneurysms, but without actual ballooning and severe dilatation of lamellar capillaries seen in microaneurysms); (4) edema and necrosis of filament epithelium; and (5) hyperplasia of the pillar cells forming the supportive framework of the lamellae, usually with thickening of the peripheral basement membrane.

Parasitic conditions--A broad spectrum of protozoan and metazoan parasites were detected in and adjacent to the gill tissue of English sole with varying degrees of host response. Due to the general non-pathogenic character of these infestations (external presence) and infections (internal presence) and the difficulty of identifying these parasites in tissue section to genus or species, taxonomic classification was usually limited to general taxonomic groups.

The most common parasite observed was the ciliate ectoparasite Trichodina sp. present adjacent to the filament and lamellar epithelium. The major host response was an excessive mucous secretion by the epithelial goblet cells when the parasite was present in denser concentrations. Generally, however, the infestation was light, with no significant lesions or host response.

An infectious condition identical morphologically to epitheliocystis in fresh water and marine fish (Hoffman et al. 1969, Zachary and Paperna 1977, Paperna 1977, Paperna et al. 1978) was found in the lamellar respiratory epithelium. The condition is an intracellular infection of epithelial cells by organisms resembling members of the Chlamydia/Bedsonia groups (Hoffman et al. 1969) and Rickettsia (Zachary and Paperna 1977, Paperna et al. 1978). Affected cells were greatly hypertrophied and contained a large cytoplasmic inclusion body consisting of a mass of uniformly sized basophilic particles. The nucleus, when visible, was greatly enlarged with a prominent nucleolus. These affected cells often reached a diameter exceeding 50 microns. The only visible host response was occasional epithelial hyperplasia encircling the infected cells in concentric layers. Heavy infections were rare. This condition is considered to be a chronic, benign, ubiquitous, infectious disease with no significant deleterious affect on the host.

A trematode (blood fluke) infection of the filaments and lamellae was also common in English sole from all regions sampled. Multiple larval forms of schistosoma-like, sanguinicolid trematodes were observed within the subepithelial connective tissue and blood vessels of gill filament shafts. These immature adults lacking reproductive organs (Chitwood and Lichtenfels 1973) elicited a variable fibrotic response in the filaments, but there was no serious pathology associated with this infection. Other parasitic conditions of the gill were as follows: (1) presence of unidentified helminth ova in the filament shaft, often with hyperplasia of surrounding chondrocytes composing the skeletal framework of the filament shaft; (2) ectoparasitism by a member of the group monogenea, possibly *Gyrodactylus sp*. (Olson 1978); and (3) infestation by copepods. None of these latter conditions were associated with any significant lesions.

Prevalence of gill lesions--

The prevalences of five of the idiopathic gill lesions and two parasitic conditions in English sole are shown in Figure 18. Respiratory epithelial hyperplasia was observed only in sole from the three urban estuaries; while lymphocytic infiltration was detected in sole from all four estuaries, but the frequencies in the urban estuaries were significantly higher than in McAllister Creek. Mucous cell hyperplasia was observed only in the Duwamish Waterway; and although microaneurysms were found in sole from two of the urban estuaries as well as the reference estuary, the prevalence in sole from Duwamish Waterway was significantly higher than that in the reference estuary. Lamellar necrosis affected only small numbers of English sole from the Duwamish Waterway and Snohomish River.

Two of the most prevalent parasitic conditions, epitheliocystis and infection by digenetic trematode larvae, were detected in English sole from all four areas. Only sole from the Duwamish Waterway had frequencies significantly higher than the sole from McAllister Creek. Trichodinids were found in the gills of sole from all sampling areas (Fig. 18).

A percentage of English sole from all areas sampled were free from microscopically detectable idiopathic and parasitic lesions in the sections examined. These "normals" (in terms of condition of the gill) were encountered most often in McAllister Creek (64.7%), followed by the Snohomish River (51.5%), Lake Washington Ship Canal (45.9%), and Duwamish Waterway (36.5%). The Lake Washington Ship Canal and Duwamish Waterway samples contained significantly fewer "normals" than the reference area ($p<0.05$ and 0.001, respectively), while the Snohomish River sample was not statistically different from the reference value. Conversely, English sole from the Duwamish Waterway and Lake Washington Ship Canal were significantly more frequently affected with idiopathic and/or parasitic conditions than English sole from either the Snohomish area or the McAllister Creek reference site.

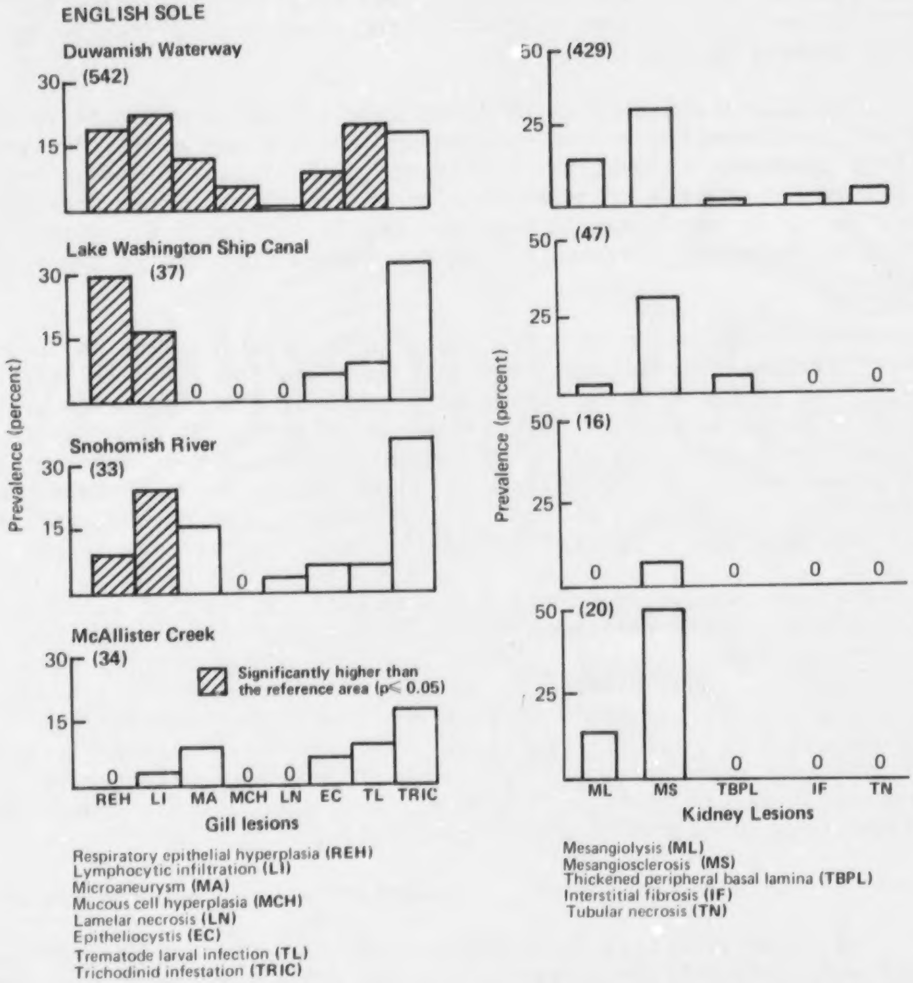

FIGURE 18. Prevalences of gill and kidney lesions in English sole from the Duwamish Waterway, Lake Washington Ship Canal, Snohomish River, and McAllister Creek sampling areas.

Characteristics and prevalence of cardiac abnormalities--

Cardiac tissues were not routinely sampled until the summer of 1979. Since only the Lake Washington Ship Canal and Duwamish Waterway were sampled in this and the winter 1980 sampling, the following descriptions will present data only from these areas, precluding other inter-area comparisons.

The most frequently encountered cardiac lesion was multifocal chronic inflammation of the myocardium (chronic myocarditis), typically with a prominent mononuclear infiltrate, significant fibrosis and occasional myocardial degeneration/necrosis. The overall prevalence for this lesion in the Duwamish Waterway was 18.9% (Table 6), with a general trend of increased prevalence from downstream stations to the upstream station.

This lesion was most often found in conjunction with an infection of sanguinicolid trematodes identical to those found in gill tissues. Occasionally, no inciting agent was visible at the light microscopic level. The prevalence of this trematode infection also tended to increase from the lower to upper Duwamish Waterway sampling stations, and showed an overall prevalence of 11.4% (Table 6). The infection was variable in severity, with significant fibrosis and chronic inflammatory cell infiltration peripheral to the parasites in the more severe cases.

Another common lesion was a non-specific idiopathic epicarditis, evidenced by multifocal aggregations of mononuclear cells in the epicardium, with increased melano-macrophage center populations, edema, and occasional congestion. The overall prevalence in English sole from the Duwamish Waterway was 7.0% (Table 6), with no general trend in the prevalences at individual stations. The etiology of epicarditis is not clear, but the occasional presence of philometrid nematodes in the pericardial cavity in English sole at gross necropsy may provide some insight into the cause of this incidental finding.

Characteristics and prevalence of gastrointestional abnormalities--

The major anomalies detected histologically in the gastrointestional tract were parasitic or parasite-related, and since the bulk of the gastrointestional tissues examined were from the small intestine immediately distal to the pyloric caeca, this section will focus on the anomalies found at that anatomical site. The most common parasitic infection was a protozoan infection by an unclassified coccidian, generally present adjacent to or embedded within the brush border of the intestinal mucosa. Often, however, histozoic forms of this undefined coccidian were present within the mucosa, and morphologies parallel to life history stages of coccidians were encountered. The host response in this infection was minimal or absent, and the agent is considered non-pathogenic. At the light microscope level this coccidian closely resembled members of the genus Eimeria, a common coccidian parasite of marine and fresh water fish (Margolis and Arthur 1979, Hoffman 1970).

TABLE 6. Lesions in selected organs and tissues of English sole.

		Prevalence (%)			
Organ	Lesion Description	Duwamish Waterway	Lake Wash. Ship Canal	Snohomish River	McAllister Creek
Heart	Chronic myocarditis	18.1(105)[a]	22.2(18)	ND[b]	ND
	Trematode infection	11.4	22.2	ND	ND
	Idiopathic epicarditis	7.0	0	ND	ND
G.I. Tract	Coccidian infection	45.0(398)	28.0(25)	11.1(18)	38.1(21)
	Nematode infection	35.9	32.0	38.9	38.1
	Acanthocephala infection	4.3	4.0	22.2	47.6
	Nonspecific mucosal necrosis	1.8	0	0	0
	Ulcerative gastroenteritis	0.8	0	0	0
Kidney	Thickening of the basement membrane	3.3(429)	6.4(17)	6.3(16)	0(20)
	Tubular protein casts	3.3	0	6.3	0
	Mesangial cell proliferation	0.5	2.1	0	0
	Myxosporidia infection	3.7	14.9	0	5.0

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[a] Number of fish examined.

[b] ND, no cardiac tissue from English sole were examined.

Prevalences of sole with coccidian infections ranged from 11.1% (2 of 18 fish) in the Snohomish River to 45% (179 of 398 fish) in the Duwamish Waterway (Table 6). By the G-statistic, the prevalence of this infection was significantly higher ($p<0.01$) in English sole captured in the Duwamish Waterway.

Another protozoan infection, confined to the intestinal lumen and also apparently non-pathogenic, was parasitism by flagellates of the Hexamita sp., a member of the order Polymastigina (Hoffman 1970). Affected English sole were found only in the Duwamish Waterway (2.5%, 20 of 398 fish).

Representatives of several helminth groups parasitized all layers of the alimentary tract wall, and were often detected within the gut lumen. The typical host response to the intramural helminths was chronic inflammation, fibrosis, and granuloma formation. Unspecified members of the class Nematoda were the most commonly encountered helminth groups, and larval and adult forms were found. The larval forms tended to be confirmed to the mucosa, lamina propria, and submucosa, while adult forms were present in the lumen, submucosa, the muscular wall, and supraserosally in the mesenteries. Undoubtedly several species were represented, with the larval forms possibly Contracaecum sp. (Olson 1978). In the study of English sole reported by Olson (1978), adult intestinal nematodes were described as Cucullanus annulatus and Thynascaris sp. Infection by acanthocephalids was also encountered in all four areas, generally with penetration of the spiny head through the mucosa and into the muscular layers, accompanied by a fibrogranulomatous response. If our findings are parallel to those of Olson (1978), the probable species is Echinorhynchus lageniformis. English sole with the above-mentioned helminth infestations were found in all of the estuaries (Table 6).

Several lesions apparently unassociated with parasites were detected only in the intestines of English sole from the Duwamish Waterway. These were found at very low prevalences (0.8 to 1.8%) (Table 6), and included non-specific necrosis of the mucosal epithelium, and three cases of chronic ulcerative gastroenteritis.

Characteristics and prevalences of renal anormalities--

Mesangiolysis--The most striking idiopathic renal condition found in the English sole kidney was a primary, degenerative, glomerular lesion which closely resembles mesangiolysis observed in humans and experimental mammals (Morita et al. 1978). This lesion was characterized histologically by varying degrees of cystic dilatation and ballooning of the glomerular tuft, loss of architectural integrity, dilation and/or congestion of the capillary component, with edema and lysis of the mesangium (the structural framework of the glomerular tuft, Fig. 19). Often there was a generalized necrosis of all glomerular tuft components, and fibrin and protein casts could be detected in the Bowman's space. In the more severe cases, only the visceral epithelium and peripheral

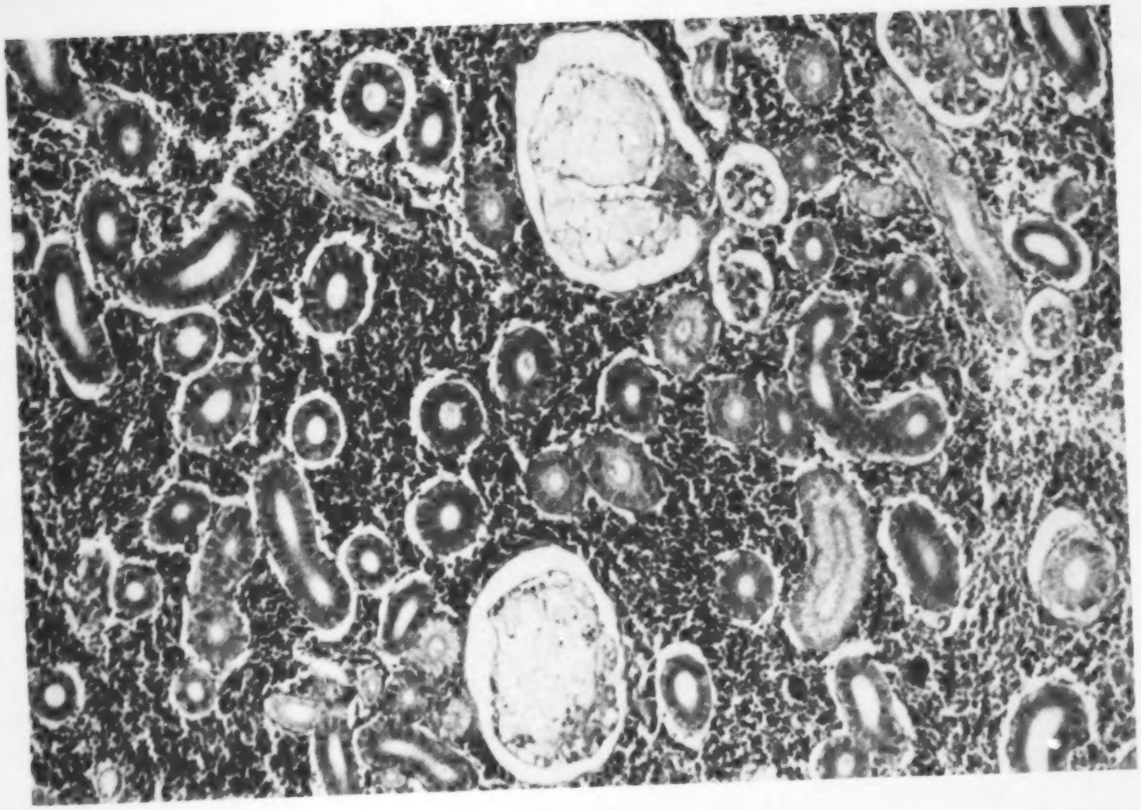


FIGURE 19. Micrograph of the renal lesion, mesangiolysis, in an English sole. This section of kidney shows two glomeruli with ballooning of the glomerular tuft and loss of mesangial architecture, both of which are characteristic of mesangiolysis. H & E, 240X.

basal lamina remained. The endothelial cells lining the capillaries were often swollen and occasionally were exfoliated. This glomerular lesion occurred in the absence of any visible tubular nephropathy, and inflammatory cell infiltrates were rarely present. Mesangiolysis was found at highest prevalences in English sole from McAllister Creek and the Duwamish Waterway (Fig. 18). No affected fish were detected in the Snohomish River. Fish as small as 100 mm were affected, but this condition was more prevalent in older fish.

Mesangiosclerosis--Mesangiosclerosis was commonly found concommitantly with mesangiolysis. Mesangiosclerosis is defined as an increase in the mesangial matrix of the glomerular tuft (Fig. 20). The matrix is composed of fine fibrils including collagen, all embedded in a mucopolysaccharide ground substance. When mesangiosclerosis and mesangiolysis were found together, one or several segments of a mesangiolytic glomerulus exhibited mesangial thickening and increased matrix density in conjunction with proliferation of mesangial cells. Rarely was the peripheral basal lamina thickened. Occasionally, the visceral and parietal epithelial components were proliferative in mesangiosclerotic glomeruli, with formation of synechiae or adhesions involving these inner and outer epithelial components of the glomerulus. Sclerosis in Bowman's capsule, peripheral to the parietal epithelium, was also sometimes observed. It is not possible to definitely prove the temporal relationship between mesangiolysis and mesangiosclerosis in a static study such as this, but principles of reaction to injury in the glomerulus suggest that mesangiolysis precedes mesangiosclerosis.

Cases of coincident mesangiolysis and mesangiosclerosis were found only in the Duwamish Waterway and McAllister Creek, representing over 20% of the total number of cases of mesangiolysis and mesangiosclerosis. Fish affected by both of these lesions also tended to be older. Using a chi-square contingency test of association, mesangiolysis and mesangiosclerosis were found to be highly associated ($p<0.001$).

Mesangiosclerosis was often detected independent of mesangiolysis, and in these cases this lesion may represent a separate pathogenetic entity. Sclerosis of the mesangium and the glomerular tuft is a common response to injury (Schillings and Stekhoven 1980) in the glomerulus and is seen in a variety of chronic renal disease states. Similar increases in mesangial matrix with mesangial cell proliferation are also associated with increasing age in humans (Wehner 1968). Mesangiosclerosis was found in all sampling areas, with highest prevalences in the Duwamish Waterway, Lake Washington Ship Canal, and McAllister Creek (Fig. 18).

Miscellaneous idiopathic renal lesions--Other renal lesions included the following: (1) thickening of the basement membrane peripheral to the neck segments and tubules, often with peritubular fibrosis; (2) degeneration and necrosis of the tubular epithelium of the first and second proximal segments of the nephron (Duwamish Waterway only, 3.3%, 14 of 429 fish); (3) thickened peripheral basal lamina in the glomerular tufts (membranous

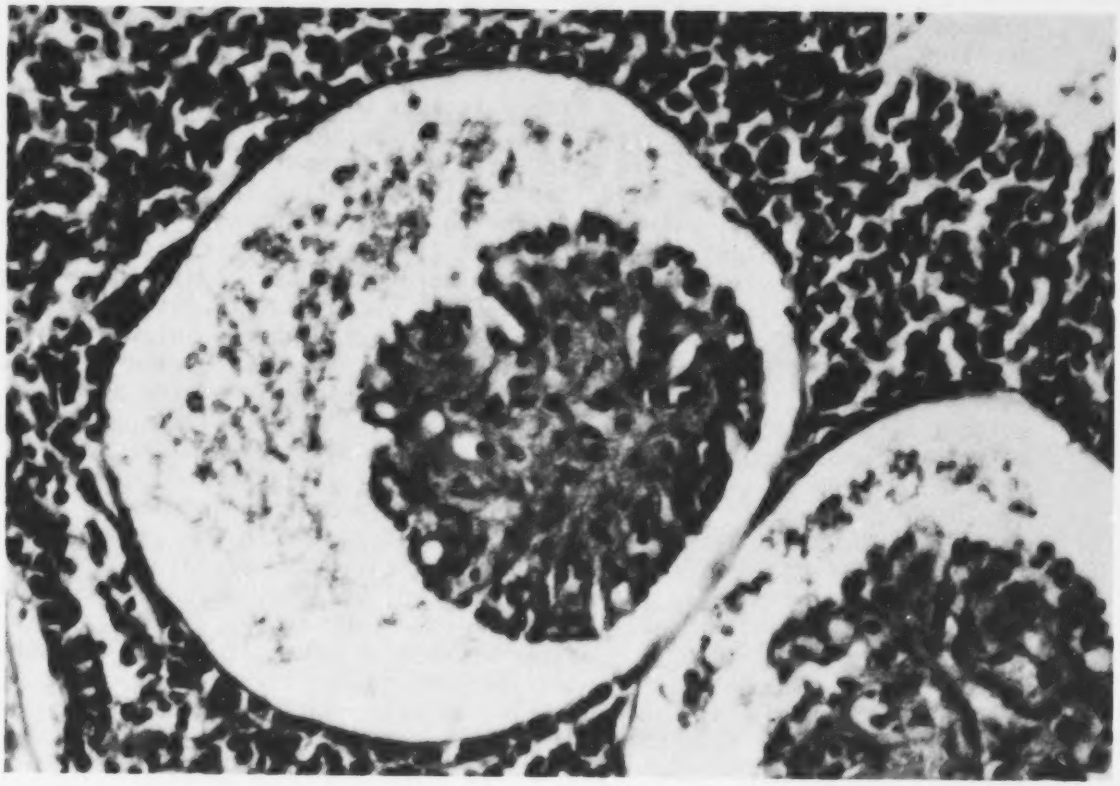


FIGURE 20. Micrograph of the renal lesion, mesangiosclerosis, in an English sole. This micrograph of a renal glomerulus demonstrates a markedly increased density in the mesangial matrix characteristic of mesangiosclerosis. H & E, 975X.

glomerulopathy, Figs. 18 and 21); (4) presence of protein casts in the renal tubular lumina (indicative of proteinuria) (Table 6); (5) mesangial cell proliferation (not in association with other renal lesions, Table 6); (6) fibrosis of the interstitial supporting framework of the kidney including fibrosis peripheral to Bowman's capsule (Duwamish Waterway only, 1.6%, 7 of 429 fish); (7) proliferation of the visceral and parietal epithelium of the glomerulus (Duwamish Waterway only, 0.7%, 3 of 429 fish); and (8) squamous metaplasia of the tubular epithelium (change from a columnar-cuboidal epithelial type to a more squamous profile) (Duwamish Waterway only, 0.5%, 2 of 429 fish).

Association of idiopathic kidney lesions with idiopathic liver lesions in English sole--Testing the hypothesis that specific idiopathic kidney lesions are associated with idiopathic liver lesions, the chi-square contigency test (Zar 1974) was applied to the combined data for all quarters, with the following results: Grouped idiopathic liver lesions are highly associated with (1) mesangiolysis ($p<0.01$); (2) mesangiosclerosis ($p<0.001$); (3) coincident mesangiolysis and mesangiosclerosis ($p<0.025$); (4) tubular epithelial necrosis ($p<0.001$); (5) tubular protein casts ($p<0.01$); (6) peritubular basement membrane thickening ($p<0.005$); (7) sclerosis around Bowman's capsule ($p<0.025$); and (8) grouped idiopathic kidney lesions ($p<0.001$). Strong associations were also evident between English sole liver neoplasms and mesangiolysis ($p<0.01$), mesangiosclerosis ($p<0.001$), coincident mesangiolysis and mesangiosclerosis ($p<0.025$), tubular eipthelial necrosis ($p<0.001$), tubular protein casts ($p<0.01$), peritubular sclerosis ($p<0.001$), sclerosis around Bowman's capsule ($p<0.025$), thickened peripheral broad lamina in the glomeular tuft ($p<0.05$), and proliferation of the parietal and visceral epithelium in the glomerular tuft ($p<0.05$). Testing the association between specific idiopathic liver and kidney lesions, MH was found to be associated with mesangiosclerosis ($p<0.001$), tubular epithelial necrosis ($p<0.001$), tubular protein casts ($p<0.001$), and fibrosis around Bowman's capsule ($p<0.01$). These results show a strong connection between renal and hepatic pathology in the English sole from the Duwamish Waterway, and the existence of potential disease syndromes manifesting themselves through associated lesions in separate organ systems.

Parasitic conditions in the kidney--The only common parasitic condition in English sole kidney was an infection of the kidney tubular lumina by an unidentified myxosporidian (Table 6).

Pathological Conditions of Starry Flounder

Characteristics and prevalence of fin and skin conditions--

The idiopathic, non-parasitic microscopic lesions included epidermal degeneration/necrosis/spongiosis, epidermal hyperplasia, subcutaneous epidermal inclusion cysts, mucous cell hyperplasia, subcutaneous hemorrhage, and subcutaneous chronic inflammation (Table 7). These lesions were detected at low prevalences almost exclusively in starry

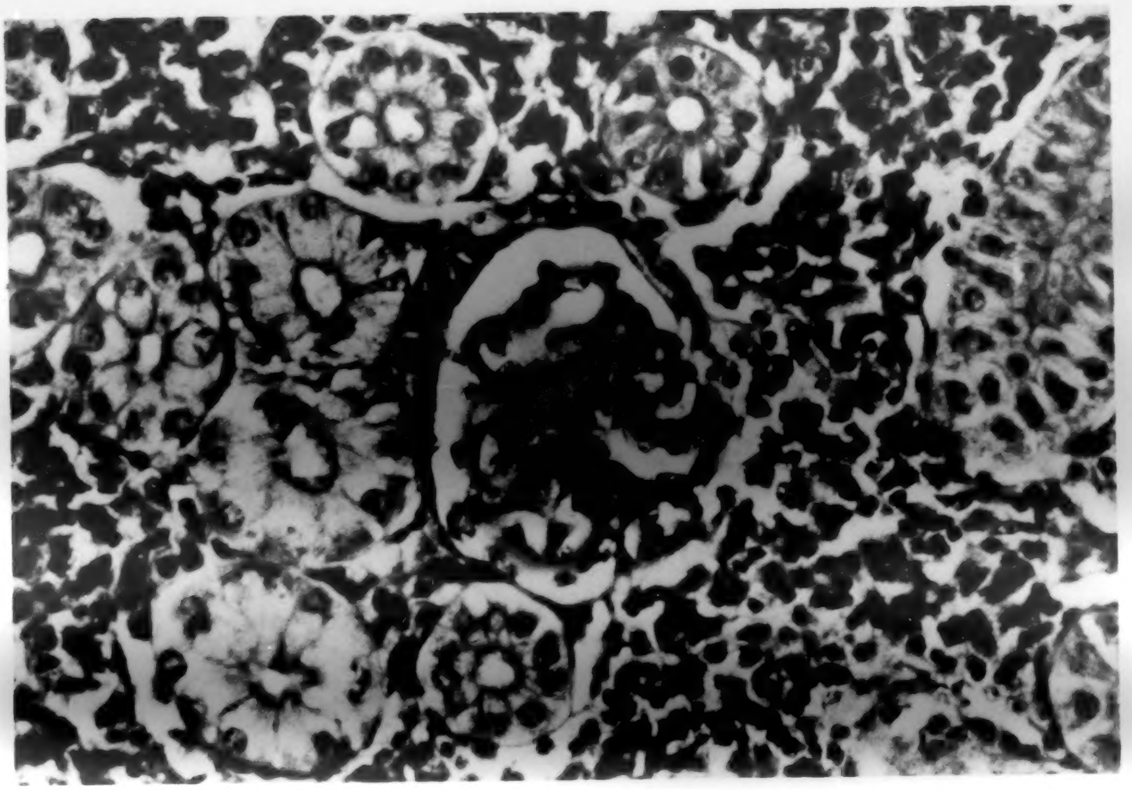


FIGURE 21. Micrograph of severe thickening of the peripheral basal lamina in the glomerular tuft in the kidney of an English sole. The renal glomerulus in the center has a severely thickened, dense peripheral basal lamina in the glomerular tuft, characteristic of membranous glomerulonephritis. H & E, 975X.

TABLE 7. Lesions in selected organs and tissues of starry flounder.

Organ	Lesion Description	Prevalence (%) Duwamish Waterway	Prevalence (%) Snohomish River	Prevalence (%) McAllister Creek
G.I. Tract	Chronic inflammation	10.2(225)*	77.8(9)	12.1(33)
	Submucosal congestion	1.3	0	0
	Coccidia infection	50.7	22.2	18.2
	Hexamita infection	2.2	0	0
	Nematode infection	14.7	44.4	18.2
	Trematode infection	2.2	11.1	3.0
	Acanthocephala infection	2.7	55.6	18.2
Kidney	Thickening of the basement membrane	0.8(243)	0(8)	3.3(30)
	Tubular protein casts	2.1	0	0
	Mesangial cell proliferation	0.4	0	0
	Myxosporidia infection	57.6	25.0	6.7
	Microsporidia infection	2.5	0	0

* Number of fish examined.

flounder from the Duwamish Waterway. A syndrome of the fins and skin, referred to as fin erosion, was found only from the Duwamish Waterway specimens, and was characterized by all or a few of the following lesion components: epidermal hyperplasia, subcutaneous chronic inflammation with fibrosis, subcutaneous hemorrhage, epidermal inclusion cysts and fin ray resorption (Wellings et al. 1976b). In cases of actual fin ray loss, the remaining unsupported fin tissue had folded back onto the adjacent skin overlying the body musculature, creating a fusional graft of tissue united by scar tissue. Microscopically confirmed cases of this syndrome were found in 2.9% (8 of 280 fish) of the flounder from the Duwamish Waterway.

Minor parasitic infestations of skin and fins were found and they consisted of subcutaneous nematodal microfilariae and an intramuscular myxosporidian infection resembling Kudoa sp.

The final lesion group has been discussed previously for English sole, namely angioepithelial nodules (AEN), transitional AEN (TAEN), and epidermal papillomas (EP). Aside from a single fish from McAllister Creek with an EP, all other specimens affected by these lesions were found in the Duwamish Waterway at the following frequencies: AEN, 1.1% (3 of 280 fish); TAEN, 0.4% (1 of 280 fish); and EP, 4.6% (13 of 280 fish).

Characteristics and prevalence of gill anomalies--

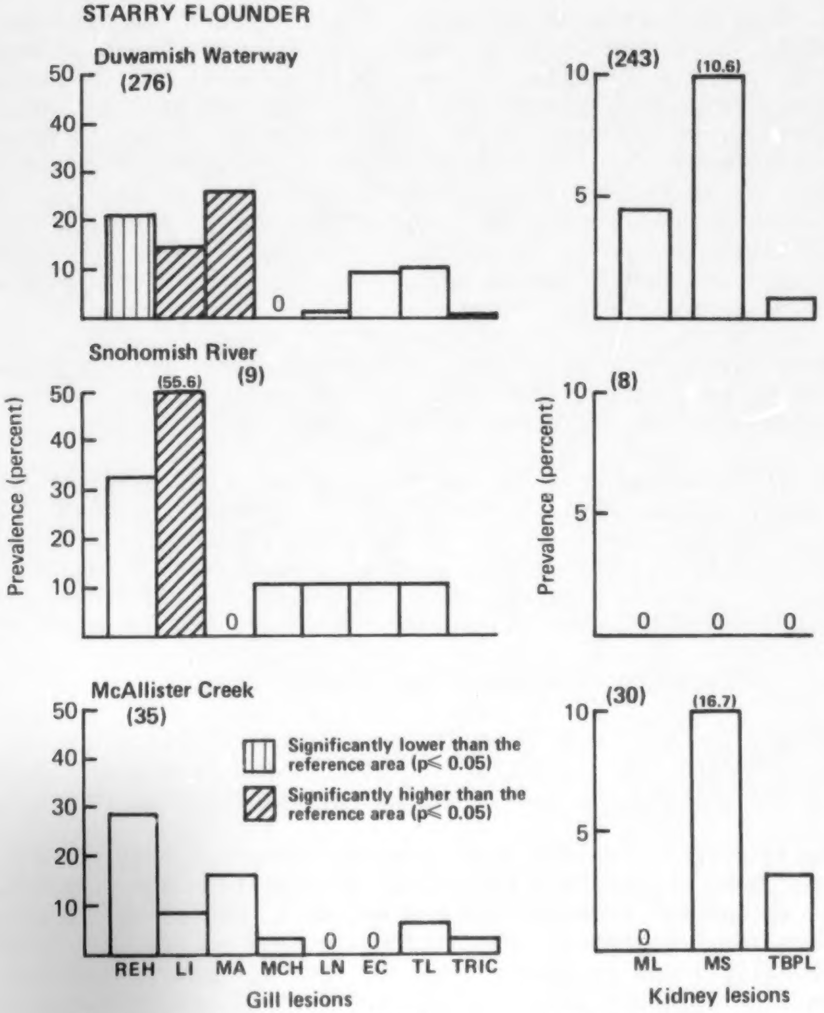
Table 7 presents the types and prevalences of parasitic and non-parasitic (idiopathic) conditions of gill tissue in starry flounder. These conditions closely resembled the lesions detected in English sole.

The idiopathic lesions (REH, lamellar lymphoid infiltrates, lamellar microaneurysms, respiratory epithelial degeneration and necrosis, filament epithelial degeneration and necrosis, pillar cell hyperplasia with increased basement membrane thickness, and mucous cell hyperplasia) were generally found in starry flounder from all sampling areas. The prevalence of flounder with lymphoid infiltrates and microaneurysms was significantly higher ($p<0.05$) in the Duwamish Waterway, compared to McAllister Creek (Fig. 22).

Flounder with infectious conditions (surface trichodiniasis, epitheliocystis, intravascular microsporidia, sanguinicolid-like digenetic trematodes and external monogenea) were found at low prevalences and were generally widely distributed (Fig. 22). The exceptions were: the absence of trichodiniasis in Snohomish River samples; absence of epitheliocystis in McAllister Creek starry flounder; presence of microsporidia only in specimens from the Duwamish Waterway; and absence of external monogenea in Snohomish River specimens.

Characteristics and prevalence of liver lesions--

Many of the same types of lesions observed in livers of English sole from the Duwamish Waterway were also found in livers of starry flounder from this estuary. Those lesions not detected in starry



Respiratory epithelial hyperplasia (REH)
Lymphocytic inflitration (LI)
Microaneurysm (MA)
Mucous cell hyperplasia (MCH)
Lamelar necrosis (LN)
Epitheliocystis (EC)
Trematode larval infection (TL)
Trichodinid infestation (TRIC)

Mesangiolysis (ML)
Mesangiosclerosis (MS)
Thickened peripheral basal lamina (TBPL)

FIGURE 22. Prevalences of gill and kidney lesions in starry flounder from the Duwamish Waterway, Snohomish River, and McAllister Creek sampling areas.

flounder included CF and HC. With the exception of the intrahepatic blood cysts (peliosis hepatis), the prevalences of all the lesions common to both species were considerably lower in starry flounder (Fig. 23). The blood cysts were found in only 0.5% (3 of 551 fish) of the English sole but in 5.0% (14 of 279 fish) of the starry flounder from the Duwamish Waterway.

Of the other three estuaries sampled in this study, sufficient starry flounder for comparative purposes were captured only in McAllister Creek. Only 3 of the 11 types of liver lesions observed in flounder from the Duwamish Waterway were also detected in this species in McAllister Creek (Fig. 23). The prevalences of two of these lesions, FC and HN, were not significantly different, while the prevalence of blood cysts in flounder from the Duwamish Waterway was significantly higher ($p<0.05$).

Characteristics and prevalence of gastrointestinal abnormalities--

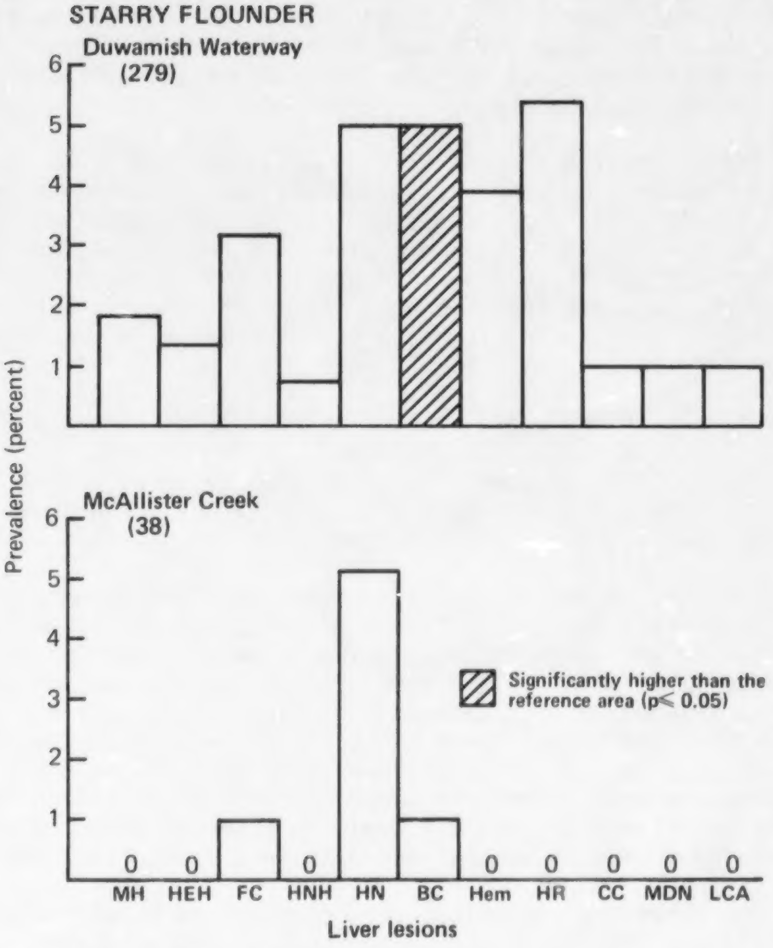
The lesions and parasitic conditions detected in the gastrointestinal tract of starry flounder were similar to those already described for English sole. Table 7 presents the types and prevalences of these conditions.

The most common non-parasitic condition was chronic inflammation with lymphoid infiltrates, fibrosis, and granuloma formation generally confined to the submucosa and lamina propria. Although this condition was not associated with visible parasitic infection, some of the cases may have represented a post-parasitic healing response. This may explain the much higher prevalence found in starry flounder from the Snohomish River; these fish also had a high prevalence of helminth infections (nematodes and acanthocephalans). The only other non-parasitic lesions were submucosal congestion (found only in the Duwamish Waterway at a low prevalence) and a single case of mucosal necrosis in a Snohomish River specimen.

The parasitic conditions were represented by protozoan (unidentified coccidia and the flagellate, *Hexamita sp.*) and helminth (nematodes, trematodes, acanthocephalans and cestodes) infections. The luminal and mucusal coccidian infection was found at a much higher prevalence in flounder from the Duwamish Waterway. *Hexamita*-infected flounder were found only in the Duwamish Waterway (Table 7). Flounder with nematode, trematode, and acanthocephala infestations were broadly distributed, but the highest prevalence was inthe Snohomish River. A single cestode infection was present in a starry flounder from the Duwamish Waterway.

Characteristics and prevalence of renal abnormalities--

The lesions encountered through histopathologic examination of kidney tissues in the starry flounder were similar to those already described for English sole. These renal lesions included mesangiolysis, mesangiosclerosis, coincident mesangiolysis, and mesangiosclerosis, thickening of basement membrane peripheral to neck segments and tubules including peritubular fibrosis, tubular epithelial degeneration and



Megalocytic hepatosis (**MH**)
Hepatocellular eosinophilic hypertrophy (**HEH**)
Fatty change (**FC**)
Hepatocellular nodular hyperplasia (**HNH**)
Hepatocellular necrosis (**HN**)
Blood cysts (**BC**)
Hemosiderosis (**Hem**)
Hepatocellular regeneration (**HR**)
Cholangiocellular carcinoma (**CC**)
Minimum deviation nodule (**MDN**)
Liver cell adenoma (**LCA**)

FIGURE 23. Prevalences of idiopathic hepatic lesions in starry flounder from the Duwamish Waterway and McAllister Creek sampling areas.

necrosis, hypermembranous glomeruli, tubular protein casts, mesangial cell proliferation (in the absence of mesangiosclerosis), and squamous metaplasia of the tubular epithelium. Mesangiolysis was found only in starry flounder from the Duwamish Waterway at a prevalence of 19.3% (47 of 243 fish, Fig. 22). However, varying degrees of mesangiosclerosis were detected in flounder in all three areas where starry flounder were captured, with the highest prevalence (37.5%, 91 of 243 fish) in the Duwamish Waterway (Fig. 22). This prevalence value was significantly higher statistically ($p<0.01$) than the prevalence values for either the Snohomish River or McAllister Creek. Coincident mesangiolysis and mesangiosclerosis were found only in Duwamish River starry flounder. By the chi-square contingency test of independence, these two lesions were found to be highly associated ($p<0.01$).

The remaining idiopathic kidney lesions were found at low prevalence and are listed by area in Table 7. Most of these lesions were detected in Duwamish Waterway starry flounder.

Parasitic infections were limited to infection of the excretory tubular lumina with unidentified non-pathogenic myxosporidia, and infection of the tubular and vascular epithelium by unidentified intracellular microsporida. The parasitic infections were found primarily in starry flounder from the Duwamish Waterway, but flounder with myxosporidian infections were found at lower prevalences in the Snohomish River and McAllister Creek (Table 7).

Ecology of English Sole and Starry Flounder

Abundance--

Catch Per Unit Effort (CPUE) is defined as the number of fish of a particular species caught in a single 5-minute trawl. At the sampling stations, CPUE values ranged from 0 to 86 for English sole and from 0 to 642 for starry flounder (Figs. 24 to 26). Average CPUE values were low for starry flounder and English sole in the Lake Washington Ship Canal and in the Snohomish River during all the sampling periods compared to CPUE values in the Duwamish Waterway and McAllister Creek.

The average CPUE values for English sole and starry flounder by season for sampling stations in the Duwamish Waterway ranged from 14.1 to 34.8 and 7.3 to 113.7, respectively (Figs. 24 and 25). The high CPUE value (113.7) for starry flounder was obtained during the winter of 1980. The Lake Washington Ship Canal, McAllister Creek, and Snohomish River were sampled during two seasons. The CPUE values for English sole for both seasons in the Lake Washington Ship Canal were 50.0 and 44.0 and in the Snohomish River the CPUE values were 12.0 and 9.8. McAllister Creek had divergent seasonal catch rates for English sole, with high CPUE values in the fall (45.0) and a nine-fold lower value (5.5) in the spring. Few starry flounder were captured in the Lake Washington Ship Canal and Snohomish River (CPUE values were less than 2.5) while moderate catch rates of this species (26.0 and 10.3) were obtained in McAllister Creek (Fig. 26).

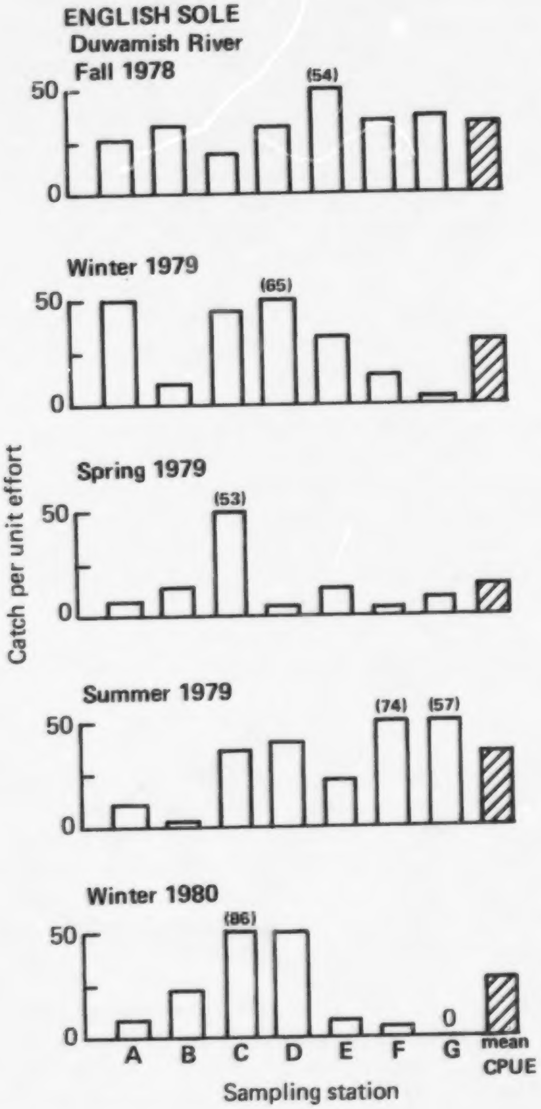


FIGURE 24. Catch Per Unit Effort (CPUE) values for English sole from the Duwamish Waterway, shown by station and sampling period.

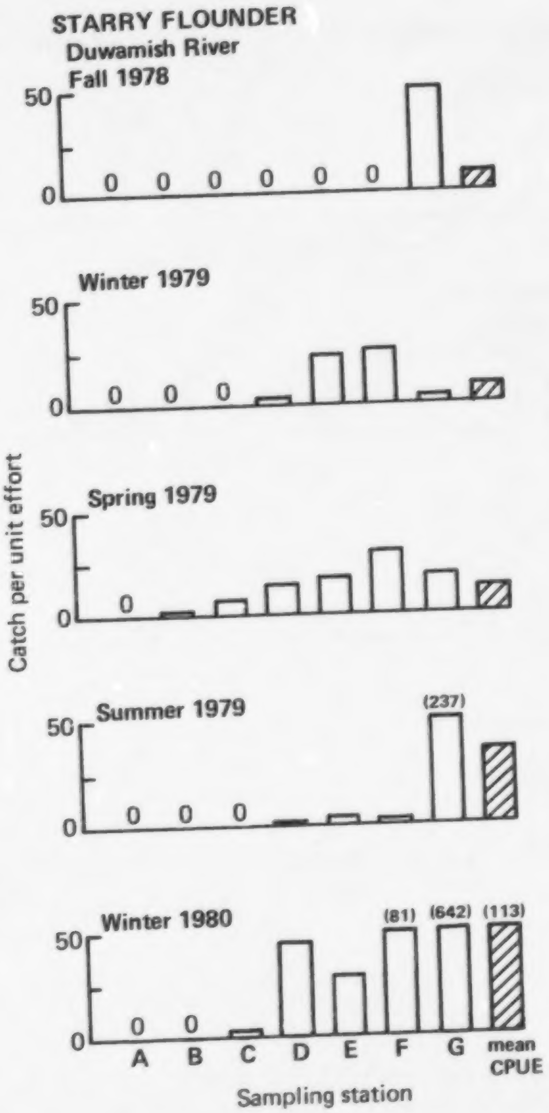


FIGURE 25. Catch Per Unit Effort (CPUE) values for starry flounder from the Duwamish Waterway, shown by station and sampling period.

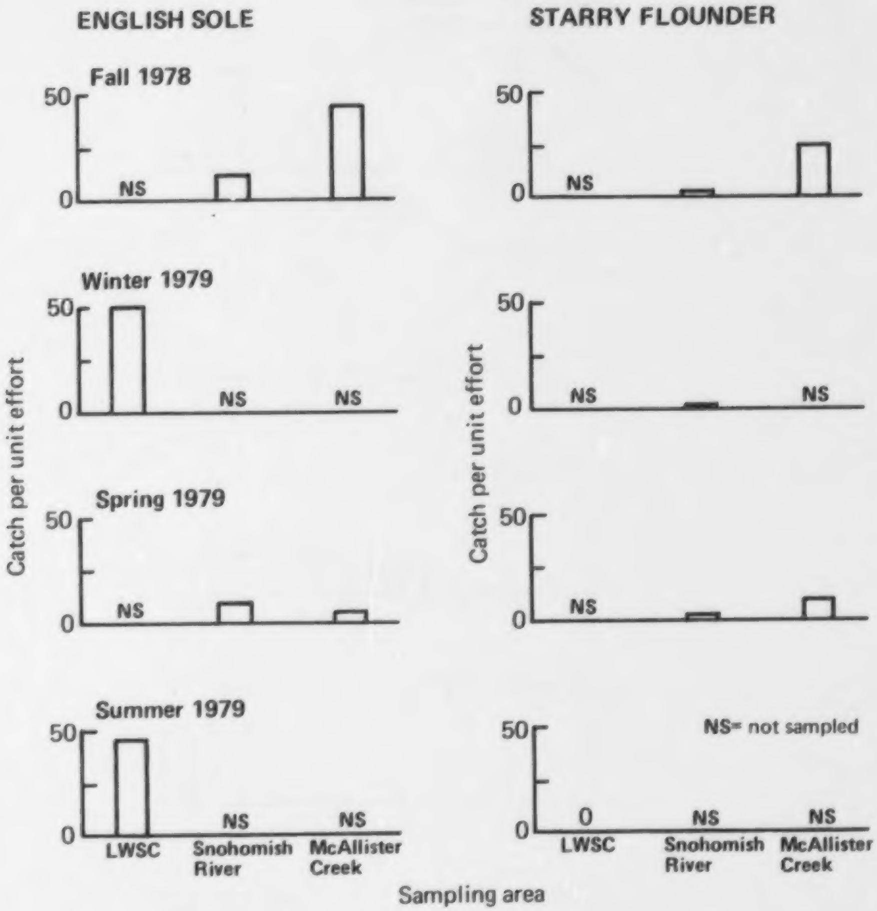


FIGURE 26. Catch Per Unit Effort (CPUE) values for English sole and starry flounder from the Lake Washington Ship Canal (LWSC), Snohomish River, and McAllister Creek sampling areas, shown by sampling period.

The geographical distribution of English sole in the Duwamish Waterway was generally even throughout the seven Waterway stations during the fall. However, few sole were captured in the upper stations during the winter and spring (Fig. 24). This distribution was reversed during the summer, with fewer English sole in the lower stations and greater abundances in the middle and uppermost stations. Starry flounder usually did not occur in the lower three Duwamish Waterway stations during any season and had differing abundances in the upper four stations (Fig. 25).

The intrusion and extent of the saltwater wedge into the mouth of the Waterway probably strongly influences the observed patterns of abundance of the target species. This dense wedge of saltwater underlies the freshwater from the Duwamish River and has an up-river boundary that depends upon the tides and the seasonal flow of freshwater from the Duwamish River drainage (Dawson and Tilley 1972). There were high catch rates of starry flounder, a species tolerant of lower salinities (Hart 1973), at the uppermost portions of the Duwamish Waterway during the summer and fall when river discharge was minimal and the saltwater intrusion was maximal. In the Waterway this species was captured almost exclusively from the uppermost portions during these seasons. The winter and spring sampling was conducted when runoff was high and flounder were commonly found at stations lower in the estuary. English sole were less abundant in stations far up the estuary during the periods of high river flow, i.e., during winter and spring, while during the summer sole were more abundant at stations in the upper estuary.

Biological characteristics--

In the Duwamish Waterway, young-of-the-year (less than 15 cm) English sole were generally most abundant during the fall, winter, and spring sampling seasons (Fig. 27). Young English sole also comprised the majority of the English sole population in McAllister Creek during the fall sampling (Fig. 28). Mid-sized English sole (15 to 24 cm) routinely comprised over half of the Snohomish River catch. Mid-sized and larger sole were in roughly equal proportions in the Duwamish River during the first four sampling quarters, but a high proportion of mid-sized fish were found in the winter of 1980. These two size groups also dominated the catches of English sole from the Lake Washington Ship Canal in the winter and summer. Mid-sized fish dominated the proportion of all the sampled starry flounder populations in the Duwamish River, but young-of-the-year starry flounder were most abundant in McAllister Creek (Fig. 29).

The presence of small target fishes in the fall, winter, and spring generally occurs with the expected sizes of young as related in Hart (1973). Starry flounder grow to approximately 10.5 cm at one year of age and English sole to approximately 15 cm. The young became available to the trawl in the fall and showed growth to the one-year-old size by the following winter.

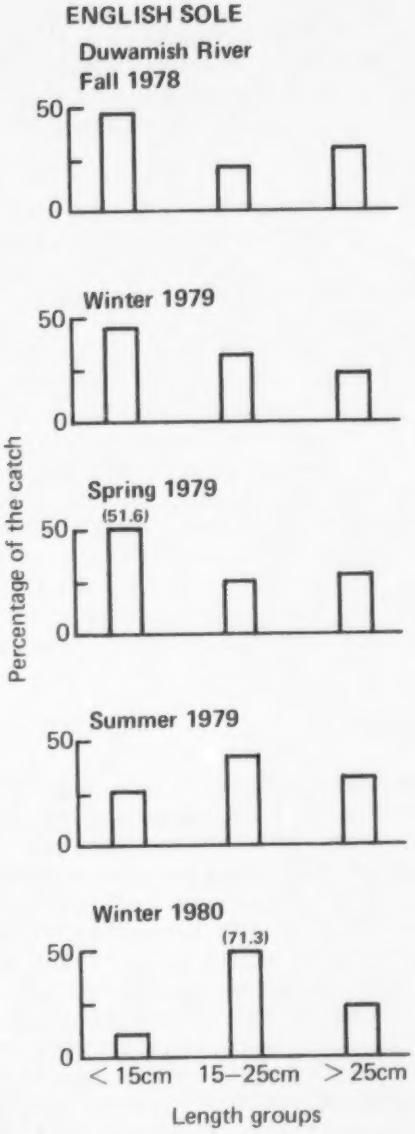


FIGURE 27. Length-frequency distributions of English sole from the Duwamish Waterway (expressed as a percentage of the catch), shown by sampling period.

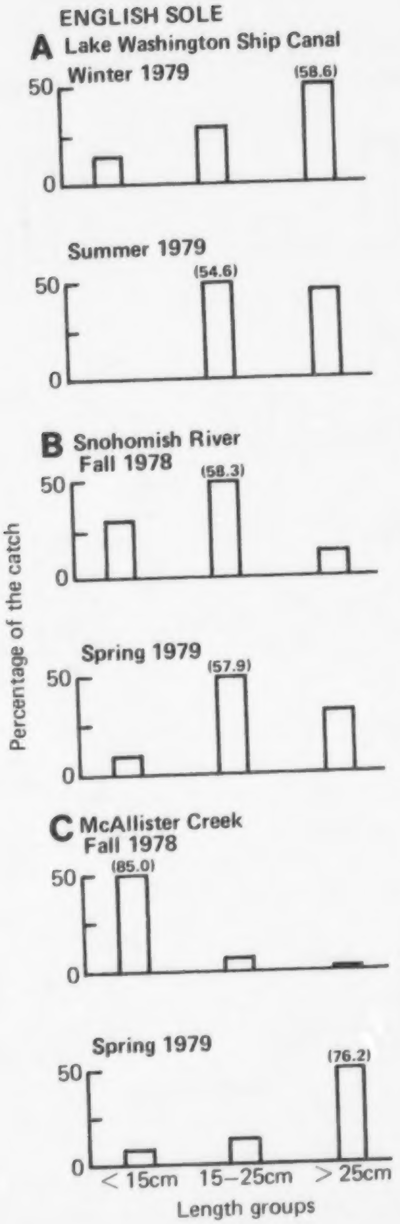


FIGURE 28. Length-frequency distributions of English sole from the Lake Washington Ship Canal, Snohomish River, and McAllister Creek sampling areas (expressed as a percentage of the catch), shown by station and sampling period.

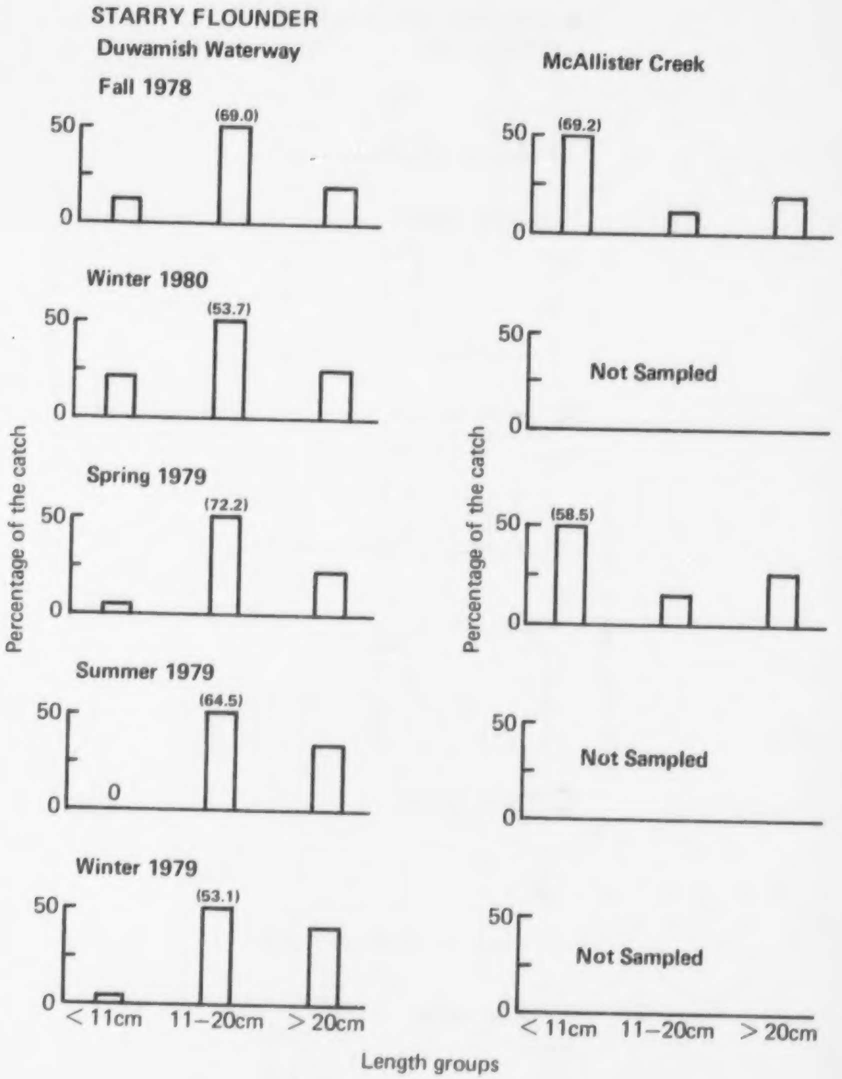


FIGURE 29. Length-frequency distributions of starry flounder (expressed as a percentage of the catch) from the Duwamish Waterway and McAllister Creek sampling areas, shown by sampling period.

Fewer juvenile English sole were captured in the winter of 1980, compared to the winter of 1979, possibly indicating a weak year class or a differential geographical distribution of young between the years. Starry flounder young were generally not as numerous in the Duwamish Waterway, compared to English sole for the same seasons. The young starry flounder may have different settling patterns than the English sole young. McAllister Creek, alternatively, had high proportions of young-of-the-year English sole in the fall, and starry flounder in the fall and spring, perhaps indicating its value as a nursery ground.

Younger (less than 15 cm) English sole and starry flounder tended to have lower prevalences of liver lesions (11% and 12%, respectively), than did mid-sized (15 to 25 cm) fish (26% and 21%, respectively) or larger (greater than 25 cm) fish (67% and 44%, respectively, Table 8). In general, the types of liver lesions observed in the younger (1 year or less in age) English sole were the degenerative types, including MH, HN, and CF (Table 9). The lesion types observed in sole as young as 2 years included CC, MDN, LCA, Hem and HEH; although the average age of fish with these lesions ranged from 5.0 to 6.2 years. No English sole younger than 3 years were observed with HNH or HC, with the average age of affected sole being 5.1 and 5.6 years, respectively.

Chemical Analyses

Chemical analyses were performed on sediment samples from all four estuaries, and on liver tissues from English sole and starry flounder from the reference estuary and the two estuaries in Seattle.

Sediment chemistry--

PCBs and selected AHs were found in all of the sediment samples from the urban estuaries (Table 10). No PCBs or the AHs listed in Table 10 were detected in sediment from McAllister Creek. The levels of sediment-associated metals also tended to be lower in the reference estuary. Within the Duwamish Waterway, the concentrations of PCBs, AHs and metals were generally highest in sediments near the mouth of the estuary and gradually decreased in sediment samples taken progressively upstream. A similar concentration gradient for PCBs in sediment within the estuary of the Duwamish River was reported by Pavlou and Hom (1979).

The concentrations of PCBs and the lower molecular weight AHs were quite similar in sediment samples from the Lake Washington Ship Canal and the Snohomish River. However, the levels of higher molecular weight AHs (those listed in Table 10 from anthracene to perylene) were considerably higher in sediment from the Lake Washington Ship Canal. In fact, the levels of these AHs in Lake Washington Ship Canal sediment were quite similar to the levels found in sediment from Station B in the Duwamish Waterway. The primary sources of these high molecular weight AHs are probably combustion products resulting from the use of fossil fuels.

TABLE 8. Prevalences of various length groups of English sole and starry flounder with liver lesions.

		English Sole			Starry Flounder		
Length Group	Age Range	No. Examined	No. Affected	Prevalence (%)	No. Examined	No. Affected	Prevalence (%)
<15cm	0 to 1	160	18	11.3	100	12	12
15 to 20 cm	2 to 3	201	52	25.8	156	32	21
>25 cm	3 to 10	192	129	67.2	34	15	44

TABLE 9. Age/length/weight characteristics of English sole with various liver lesions.

Lesion Type	Number of Fish	Average Length	Length Range	Average Weight	Weight Range	Average Age	Age Range
Megalocytic hepatosis	67	243.2	101-402	167.6	8-550	4.0	1-10
Eosinophilic hypertrophy	40	306.2	215-402	267.6	100-550	6.2	2-13
Fatty Change	36	304.4	130-405	268.9	18-620	5.5	1-10
Nodular hyperplasia	7	302.3	220-350	245.3	100-355	5.1	4- 7
Hepatocellular necrosis	50	269.1	91-442	213.9	5-620	4.7	1-10
Hemosiderosis	22	310.1	195-402	269.8	55-550	6.0	2-13
Hepatocellular regeneration	5	273.8	168-330	195.2	48-363	4.8	1- 8
Cholangiocellular carcinoma	10	280.7	175-360	206.6	41-390	5.0	2- 8
Minimum deviation nodule	19	303.6	175-402	272.0	41-550	5.7	2- 9
Liver cell adenoma	32	299.8	166-442	256.2	39-502	5.7	2-10
Hepatocellular carcinoma	23	321.8	230-402	303.8	109-550	5.6	3- 9
Mixed carcinomas	6	314.0	275-374	270.0	170-450	5.0	4- 6

TABLE 10. Concentrations of chemicals in sediment at sampling stations.

Compound or Element	Duwamish River							Lake		
	A[a]	B[b]	C	D	E	F	G	Washington Ship Canal	Snohomish River	McAllister Creek
				(ppm dry wt)						
Total PCBs	0.65	0.53	0.18	0.40	0.29	0.18	0.05	0.06	0.07	<0.01
Naphthalene	0.31	0.08	0.04	0.06	0.04	0.03	0.01	0.11	0.30	<0.01
2-Methylnaphthalene	0.15	0.06	0.05	0.08	0.05	0.05	0.03	0.05	0.09	<0.01
1-Methylnaphthalene	0.09	0.03	0.02	0.03	0.02	0.03	0.01	0.03	0.05	<0.01
2,6-Dimethylnaphthalene	0.08	0.05	0.05	0.13	0.04	0.03	<0.01	0.04	0.02	<0.01
Fluorene	0.35	0.17	0.05	0.10	0.05	0.03	0.03	0.04	0.05	<0.01
Dibenzothiophene	0.14	0.08	0.08	0.07	0.04	0.05	0.04	0.05	0.04	<0.01
Phenanthrene	2.60	0.96	0.33	0.60	0.40	0.10	0.27	0.57	0.25	<0.01
Anthracene	0.63	0.29	0.10	0.19	0.11	0.02	0.04	0.18	0.02	<0.01
Fluoranthene	3.70	1.70	0.72	0.73	0.79	0.15	0.15	0.97	0.45	<0.01
Pyrene	3.20	1.20	0.40	0.46	0.45	0.13	0.34	1.60	0.33	<0.01
Benz[a]anthracene	2.70	1.10	0.56	0.52	0.30	0.13	0.29	1.10	0.15	<0.01
Chrysene	2.40	0.92	0.42	0.40	0.37	0.10	0.24	0.75	0.15	<0.01
Benz[e]pyrene	1.70	0.48	0.25	0.24	0.20	0.05	0.12	0.55	0.04	<0.01
Benz[a]pyrene	1.50	0.44	0.19	0.19	0.18	0.04	0.27	0.63	0.03	<0.01
Perylene	0.53	0.27	0.20	0.13	0.21	0.11	0.30	0.30	0.02	<0.01
Silver	2.5	3.0	2.4	1.6	2.3	1.5	2.3	2.0	ND[b]	ND
Beryllium	0.9	1.0	0.8	0.6	0.8	0.5	0.8	0.4	ND	ND
Cadmium	11.3	11.9	10.0	7.0	8.6	5.7	8.6	5.6	ND	<0.1
Chromium	63.9	44.8	46.0	33.7	39.4	29.0	37.3	48.0	ND	13.4
Mercury	0.8	0.4	0.3	0.3	0.2	0.1	0.2	0.4	ND	0.02
Nickel	38.9	36.0	35.6	28.4	30.6	23.7	30.0	46.7	ND	ND
Phosphorous	1020.0	1174.0	1286.0	881.0	1376.0	898.5	133.3	640.5	ND	ND
Antimony	80.4	73.4	79.6	54.2	67.7	40.8	67.9	34.7	ND	ND
Strontium	123.0	124.0	130.3	92.4	121.5	79.4	131.0	59.6	ND	ND
Vanadium	92.2	95.9	98.0	82.3	88.0	68.6	90.8	58.7	ND	ND
Cobalt	13.5	17.3	21.4	14.0	16.2	10.6	14.3	9.1	ND	ND
Copper	206.0	131.0	116.0	52.4	67.0	38.3	59.7	50.5	ND	9.2
Lead	627.0	265.0	154.0	63.2	92.5	30.3	77.0	60.9	ND	38.0
% Carbon	1.8	1.8	2.4	2.0	2.9	1.5	2.9	1.7	ND	ND
Sand/Mud Ratio	0.22	0.18	0.13	0.61	0.16	1.88	0.12	0.34	ND	ND

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[a] Chemistry data for sediment samples from stations A and B were obtained as part of a companion study (Malins et al. 1980).

[b] ND, not determined.

Tissue chemistry--

The concentrations of PCBs in liver tissues clearly corresponded to the levels in the sediment from which the animals were captured (Figs. 30 and 31). No such correspondence was observed with AHs or metals. Regardless of the concentrations of AHs in the sediment, levels in liver tissue were always very low or not detectable. The most likely explanation for this observation is that even though English sole and starry flounder can readily take up AHs from the sediment (McCain et al. 1978b, Varanasi and Gmur 1981b), these species rapidly convert these AHs to oxygenated compounds which are not detectable by routine gas chromatographic techniques (Malins et al. 1979, Varanasi et al. 1979). Naphthalene (NPH) was the only AH that was routinely detected in liver tissue of English sole from the Duwamish Waterway and the Lake Washington Ship Canal. Two composite samples of liver tissue from English sole taken from the Lake Washington Ship Canal had concentrations of NPH of 0.06 and 0.04 μg/g (dry weight). In the Duwamish Waterway, English sole had NPH concentrations ranging from 0 to 0.48 μg/g (dry weight). The tendency for NPH to be present in English sole livers while other aromatic hydrocarbons are not detectable has also been reported by Varanasi and Gmur (1981a). They found that NPH taken up from contaminated sediment was metabolized at a much slower rate than was another AH, BaP.

The lack of correspondence between tissue levels and sediment levels of metals may be due largely to the varying degrees of bioavailability of many of the metals, or may be due to differential excretion. Because a weak acid was used to extract the sediment samples, the concentrations of sediment-associated metals measured in the extract do not necessarily reflect the levels of metals that are bioavailable to these fish species.

Interrelationships between chemistry and pathology--

Interrelationships between the occurrence of pathological conditions in flatfish and the presence of xenobiotic chemicals in their environment were reflected in the levels of chemicals in the tissues of fish with certain types of lesions and/or in the levels of chemical contaminants in sediments compared to the prevalence of fish with lesions. An example of comparing lesions and tissue xenobiotic levels is demonstrated in Figure 31. No consistent pattern was observed in the tissue concentrations of PCBs and NPH in liver tissues of English sole with a variety of liver lesions.

The second type of approach in evaluating interrelations between chemistry and pathology involves comparing lesion prevalence with sediment chemistry. Since English sole from the Lake Washington Ship Canal and the Duwamish Waterway had similar prevalences of liver neoplasms, 8.2% (4 of 49 fish) and 12.7% (70 of 551 fish), respectively, it is of interest to compare the chemical composition of the sediment samples from both estuaries. The concentrations of aromatic hydrocarbons and

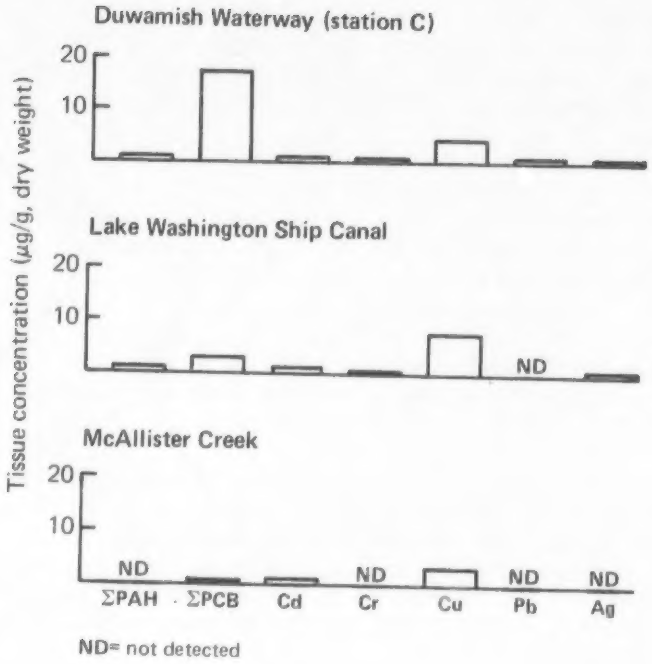

FIGURE 30. Concentrations of toxic chemicals in liver tissue from normal-appearing English sole from the Duwamish Waterway, Lake Washington Ship Canal, and McAllister Creek.

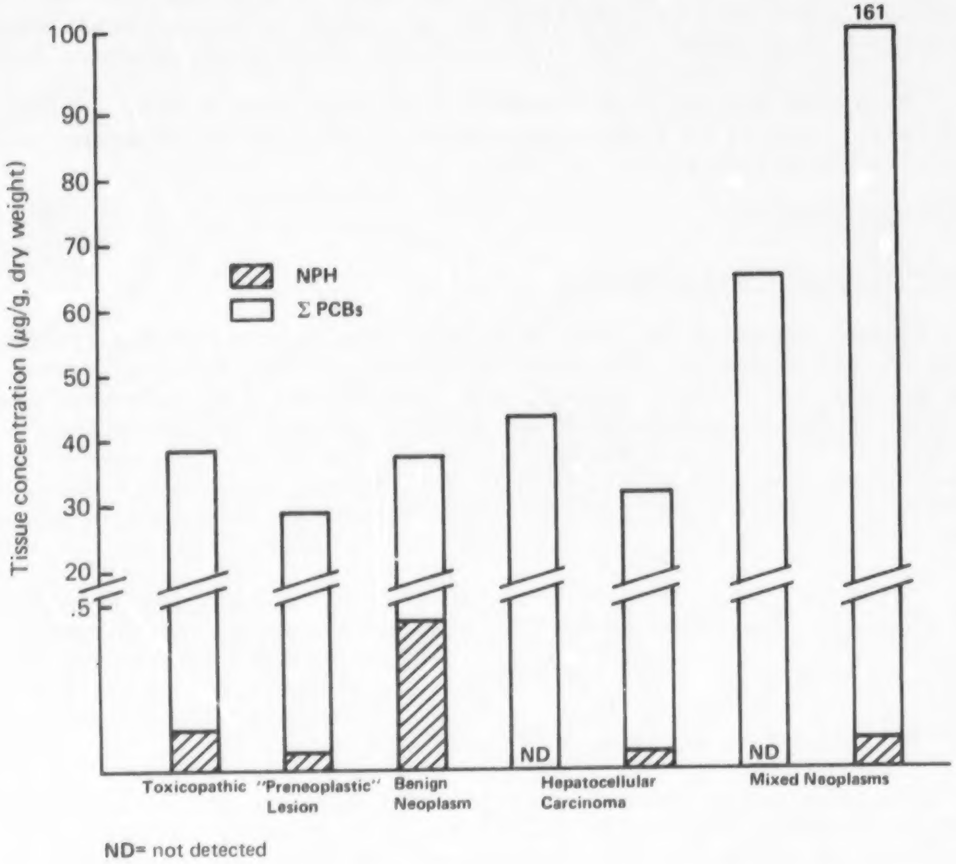

FIGURE 31. Tissue concentrations of naphthalene (NPH) and Σ polychlorinated biphenyls (PCBs) in English sole with various liver lesions.

metals in sediment in the Lake Washington Ship Canal were quite comparable with the concentrations in sediment from many of the sampling stations in the Duwamish Waterway (Table 10). However, with the exception of sediment from Station G in the Waterway (a site that is annually dredged), the levels of PCBs in the Lake Washington Ship Canal sediment were 3 to 11 times lower than in sediment from the Duwamish Waterway. Concentrations of PCBs in the livers of sole from the Lake Washington Ship Canal were generally 5 to 20 times lower than in livers of Duwamish Waterway sole.

These data suggest that exposure of English sole to PCBs may not be directly related to liver neoplasms or other types of liver lesions in English sole such as HEH and MH.

LABORATORY STUDIES

Exposures to Sediment Extracts

In experiments in which English sole were injected with extracts of bottom sediments from the Duwamish Waterway, these sediments were more acutely toxic than sediments from Port Madison (the reference area). The results are consistent with the finding of lower concentrations in the sediment from Port Madison (Table 3) of most of the chemical compounds analyzed. However, none of the histopathological conditions observed in English sole from the Duwamish Waterway were seen in injected fish.

The failure to induce the same hepatic lesions seen in Duwamish Waterway English sole may be because a longer exposure time is needed to produce these lesions. Of course a number of other factors may be responsible for this inability to induce the lesions seen in the field, including: 1) exposure by injection does not readily induce these lesions, and 2) fish were not of a susceptible age.

In a preliminary experiment (Experiment One), no mortalities were observed in groups injected with Port Madison extracts, and although mortalities were seen in fish injected with Duwamish River sediment extract, the small number of fish injected probably accounted for the finding of no statistically significant difference between the two groups. In Experiment Two, significantly more of the fish injected with extracts from Duwamish River sediments died than did those injected with corn oil ($p<0.001$), Port Madison sediment extracts ($p<0.005$), or uninjected control fish. Although Experiments One and Two are not directly comparable because the sediment extracts used in Experiment Two were about three times more concentrated than the extracts used in Experiment One, they both demonstrated the acutely toxic effects of Duwamish River sediment.

Histopathological changes were observed in the livers of sole injected with extracts of either test or reference sediment (Table 11). The changes were minor alterations in the appearance of hepatocytes; one included a finely granular (rather than the usually-observed coarsely

TABLE 11. Effects on English sole of injections of extracts of bottom sediments.

Experimental Parameter	Duwamish River Extract		Port Madison Extract		Controls	
	Experiment One	Experiment Two	Experiment One	Experiment Two	Experiment One	Experiment Two
MORTALITY						
Dilutions of Extract						
Undiluted	-	100%(5/5)[a,al]	-	40%(2/5)	-	-
1/2	66%(2/3)	100%(5/5)[a,al]	0%(0/3)	25%(1/4)	-	-
1/10	-	20%(1/5)	-	0%(0/5)	-	-
1/20	33%(1/3)	-	0%(0/3)	-	-	-
Corn oil only	-	-	-	-	0%(0/3)	0%(0/5)
No injection	-	-	-	-	ND[b]	0%(0/5)
HISTOPATHOLOGY						
Granular Hepatocellular Cytoplasm (GHC)						
Hepatocellular Hypertrophy (HH)						
Undiluted and 1/2	GHC 100% (1/1)[f] HH 100% (1/1)	No Survivors	GHC 50% (1/2) HH 0% (0/2)	83% (5/6) 50% (3/6)	- -	- -
1/10[c] or 1/20[d]	GHC100% (2/2)[f] HH 0% (0/2)	50%(2/4) 50%(2/4)	GHC 50% (1/2) HH 0% (1/2)	0% (0/5) 0% (0/5)	- -	- -
Corn oil only	-	-	-	-	GHC 0%(0/2) HH 0%(0/2)	0%(0/5) 0%(0/5)
No injection	-	-	-	-	ND	0%(0/5) 0%(0/5)
HEMATOCRIT						
Undiluted	ND	No survivors	ND	8.3 ±3.7(4)[a]	-	-
1/2	10.0%(1)[e]	No survivors	26.6(2) ±5.9	15.2(3) ±3.3	-	-
1/10[c]	ND	17.4(4) ±1.9	ND	16.0(3) ±0	-	-
1/20[d]	21.05(2) ±5.7	ND	21.8(2) ±1.8	ND	-	-
Corn oil only	-	-	-	-	23.3(2) ±0.4	21.3(4) ±6.4
No injection	-	-	-	-	ND	23.0(4) +4.2

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[a] Significantly different from control values.

[al] Significantly different from Port Madison values.

[b] ND, not done.

[c] Fish were injected with a 1/10 dilution only in Experiment Two.

[d] Fish were injected with a 1/20 dilution only in Experiment One.

[e] Number of fish from which blood samples were tested are in parentheses.

[f] Significantly different from control values (note that dilution groups were combined).

- Not applicable.

flocculent) cytoplasmic texture (GHC), and the other change consisted of slight hepatocelluar hypertrophy (HH) (Table 11). In Experiment One, granular changes in the hepatocellular cytoplasm were observed in fish injected with Duwamish or Port Madison sediment extracts, with no changes seen in either control group. Hepatocellular hypertrophy was seen in only one fish injected with a 1/2 dilution of Duwamish River sediment extract. In Experiment Two, the granular hepatocellular changes were observed in both Duwamish and Port Madison sediment extract-injected fish, with hepatocellular hypertrophy also seen in the same groups. No hepatocellular changes were found in the control groups. Experiment Three showed fish with granular cytoplasmic changes only in the group injected with Duwamish River sediment extracts (Port Madison extracts were not tested) with no changes in the livers of fish from control groups.

In analyzing these results (Table 11) statistically by the chi-square contingency test, the data for dilution groups was combined for each general group (i.e., 1/2 dilution combined with 1/10 or 1/20 for Duwamish River sediment extract-injected fish) and then the three general groups (Duwamish River, Port Madison, and corn oil-injected controls) were compared to test the null hypothesis that the presence of hepatic anomalies is independent of treatment group. In Experiment One, granular hepatocellular changes (GHC) were found at a significantly higher frequency by this test than in the corn-oil injected controls. No significant differences were found either between Duwamish River sediment-injected fish and Port Madison test fish or Port Madison and control fish affected with GHC. Hepatocellular hypertrophy (HH) was independent of treatment group. Both HH and GHC were independent of treatment group in Experiment Two. No other significant differences were found among the groups tested. The importance of granular hepatocellular cytoplasmic changes is not known, but it may represent an early degenerative change. However, the inconsistencies among the three experiments with regard to this subtle histological change makes it impossible to make any conclusions as to the toxicity of Duwamish River sediment to English sole liver relative to that of the Port Madison sediment.

Another histological change, characterized as chronic visceral inflammation, was observed in most of the sole injected with either sediment extracts dissolved in corn oil or with corn oil alone. Chronic inflammation was observed most frequently in the mesenteries and the stroma (connective tissue portion) of the pancreatic tissue scattered throughout the mesenteries, but this response was also observed with a lesser frequency in the serosal surfaces of both the liver and intestinal tract. The inflammatory response was characterized by a marked mononuclear cell infiltrate with or without associated fibrosis, or necrosis, or both. This condition therefore appears to be a response to the i.p. injection of corn oil.

In an additional experiment, English sole were injected with extracts of interstitial water from either Duwamish River or Port Madison sediment. Fish injected with the test extracts did not have significantly higher mortality, lower hematocrits, or frequencies of hepatic lesions than did the fish receiving the control extracts.

In one sediment-extract injection experiment (Experiment Two), mortality was the most important assessed parameter, where a statistically significant difference was observed between fish injected with extracts of Duwamish River test sediment and those injected with extracts of control sediment or with corn oil. Mortality results suggested the presence of an acutely toxic substance (or substances) in the test sediment, but further exposure studies would be required to confirm this single finding and identify the responsible toxicant(s). Furthermore, a significant difference in mortality between the two sediment types was only observed when the fish were injected twice with relatively undiluted preparations of the sediment extract which was concentrated three-fold suggesting that the toxicant(s) was present in relatively low levels in the original sediment. Alternatively, the toxicants may have been detoxified by the extraction procedure, or the toxicity may have been reduced during storage of the extract.

Exposures to Bottom Sediments

For Experiment One, mortality in the test (Duwamish River) sediment group was 16% (16 of 100 fish), while that in the control (Snohomish River) sediment group was 27% (27 of 100 fish, Table 12). Statistical tests indicated that mortality was independent of the sediment type used for the exposure ($\chi^2=3.58$, $p=0.059$). No consistent histopathological or hematological differences between the test and control groups were observed through Day 92, the termination date.

Chemical analysis of livers from fish exposed to Duwamish River and Snohomish River sediments for approximately two months revealed similar levels of PCBs in test (2.8 µg/g dry wt) and control (1.4 µg/g dry wt.) fish. No AHs were detected in the livers of either groups of fish. The concentrations of PCBs and AHs in Snohomish River sediment were considerably lower than in sediment from the Duwamish Waterway.

Mortality for Experiment Two was 54% (19 of 35 fish) in the test (Duwamish Waterway) sediment group and 38% (12 of 32 fish) in the control (Port Madison) group prior to termination of the test. Mortality was independent ($\chi^2=1.89$, $p=0.169$) of the type of sediment to which the fish were exposed. Microscopically, no differences between tissues of test and control animals were observed through Day 65, the last sampling date. Hematocrit hemoglobin values for test and control fish were not consistently different.

The purpose of these laboratory experiments was to compare histological and hematological findings for fish exposed to sediments and interstitial water from polluted (test) and presumed non-polluted

TABLE 12. Mortality after exposure of English sole to bottom sediments.

	Value of Parameter		
Experimental Parameter	Duwamish Waterway	Snohomish River	Port Madison
Mortalities			
Experiment 1 (after 92 days)	16%(16/100)	27%(27/100)	
Experiment 2 (after 65 days)	54%(19/35)		38%(12/32)

(control) areas. Nevertheless, the control sediments chosen for the study contained certain pollutants which may have affected experimental results. For example, the Port Madison sediment contained a higher concentration of chlorinated butadienes than did the Duwamish River sediment (Table 3). Additionally, Snohomish River sediment contained relatively high levels of pesticides and PCBs as indicated by the concentrations detected in fish which had been exposed to this sediment for approximately 2 months. If possible, a control sediment lacking in such pollutants should be used if further sediment exposure experiments are conducted. It may be necessary to search outside of Puget Sound for an ideal control sediment.

None of the characteristic lesions observed in English sole from the Duwamish Waterway were seen in fish in these laboratory experiments. One possible explanation for this results was the relatively short duration of the exposures.

The average age of Duwamish River English sole exhibiting pollution-associated hepatic lesions in the field portion of this study was 4.54 ± 2.46 years, which is consistent with an extended period of exposure to a particular pollutant or combination of pollutants occurring prior to development of the lesions. The longest laboratory exposure to any test or control substance was 92 days.

Benzo[a]pyrene Bioavailability Experiment

Blood, liver, muscle and bile of English sole contained considerable radioactivity at 24 hours after the fish were placed on sediment (Table 13). Bioconcentration values of BaP in tissues were calculated using both the concentration of BaP in sediment and sediment-associated water (SAW). The bioconcentration value for liver was greater than that for either blood or muscle. Liver and muscle contained 410 and 5.7 times, respectively, as much BaP-derived radioactivity as that present in the SAW. The bioconcentration in the liver was also higher than for blood or muscle when the bioconcentration factor was based on sediment concentrations.

BaP was extensively metabolized and excreted in the bile as evidenced by large concentrations of BaP-derived radioactivity in bile (Table 13) and the presence of high proportions of metabolites in bile of these fish (Table 14). Of the total radioactivity in bile, 94% was present as BaP metabolites. Enzymatic hydrolysis revealed that 26% of the radioactivity in bile was in the form of glucuronic acid conjugates of BaP. Low specific activity of BaP prevented further analyses of the samples to identify individual BaP metabolites in either liver or bile of these fish.

This experiment clearly demonstrated that BaP associated with sediment is bioavailable to English sole. Uptake of sediment-associated BaP can occur via the gills or via the gastrointestional tract as a result of fish contacting and ingesting particle-bound BaP together with solubilized BaP present in SAW. Earlier results reported by

TABLE 13. Concentrations of benzo[a]pyrene (BaP)-associated radioactivity in sediment, sediment-associated water (SAW) and tissues of English sole[a], and the bioconcentration factors for the tissues relative to BaP concentrations in the sediment and SAW.

Time of Exposures (hours)	Sediment (pmoles/g)	Sediment-Associated Water Unfiltered (pmoles/ml)	Blood	Liver	Muscle	Bile
			(pmoles BaP/g tissue dry wt.)			
0	2200±320	12±10	c	c	c	c
24	2700± 97	6±5	390±50	2400±1200	34±18	31,000±17,000
		Bioconcentration Factors				
24	c	c	0.15[d]	0.92	0.13	12
			66[e]	410	5.7	5100

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[a] Sediment and SAW samples were taken from the exposure tanks as described in the text. SAW was decanted from the sediment and analyzed unfiltered. Three replicates of sediment were analyzed to get average amount of tritium in each sample.

[b] Concentration values are reported as the mean ± SD.

[c] Not done.

[d] Based on sediment concentrations.

[e] Based on SAW concentrations.

TABLE 14. Proportions of the metabolites of benzo[a]pyrene (BaP) in bile of English sole exposed to contaminated sediment.[a]

Percent of total radioactivity			
Before enzymatic hydrolysis[b]		After enzymatic hydrolysis[c]	
Ethyl actetate phase	Aqueous phase	Ethyl acetate phase	Aqueous phase
6	94	26	74

[a] Two samples of bile from separate fish were pooled.

[b] Bile was extracted with ethyl acetate to separate unconverted BaP and its non-conjugated metabolites from the conjugated metabolites.

[c] The remaining aqueous phase containing the conjugated metabolites was treated with Glucurase and hydrolyzed. HPLC analysis was performed on the ethyl acetate fraction but no conclusive results were reported due to low activity of the sample.

Varanasi and Gmur (1981a), in which English sole were exposed to BaP in oiled sediment, showed that BaP in SAW was associated mainly with the particles. Lakowicz et al. (1980) reported that adsorption of BaP to particulates results in an increased rate of membrane uptake compared to that obtained with an aqueous suspension of BaP. The results of Martin (1980) suggest that uptake of BaP via the gastrointestinal tract in estuarine fishes may be the major route.

Large proportions of BaP metabolites in bile of English sole indicate that these fish metabolize BaP and excrete BaP-derived compounds efficiently. BaP, administered orally, is also metabolized extensively by pleuronectid fish (Varanasi and Gmur 1981b). Moreover, as with earlier studies (Varanasi et al. 1979, Varanasi and Gmur 1981a, 1981b) in which bottom fish were force fed either naphthalene or BaP, glucuronides were the major class of conjugates present in the bile. Glucuronidation is also identified as a major detoxification pathway for polynuclear aromatic hydrocarbons in mammals (Baird et al. 1977).

In this study, individual metabolites of BaP were not characterized in liver or bile because of the low levels of radioactivity. However, in a separate study (Varanasi and Gmur 1981b), BaP is biotransformed by English sole liver into a number of metabolites which are known to be mutagenic and carcinogenic in mammals (Brookes 1977). Notable among these metabolites was 7,8-dihydro-7,8-dihydroxy BaP, a proximate carcinogen. Studies with mammals (Sims et al. 1974) show that this dihydrodiol is further metabolized to produce a diolepoxide which binds covalently to DNA, and that such chemical modification of DNA is implicated as the critical step in a series of events leading to some chemically-induced neoplasms. In a recent report (Varanasi et al. 1981), it was shown that when BaP is force fed to English sole, a significant portion of the BaP intermediates become covalently bound to liver DNA. In view of these findings, the present results showing the ability of English sole to take up and bioconcentrate sediment-associated BaP may have considerable environmental significance.

APPENDIX I

Lymphocystis Disease

Lymphocystis disease in marine fishes of the eastern Pacific Ocean was reported in yellow fin sole (Limanda aspera) from the Bering Sea (Alpers et al. 1977a); in three species of Sebastes from the Steinhart Aquarium in San Francisco (McCosker et al. 1976); in Hypsoblennius spp. commonly found in intertidal and subtidal waters in bays of southern California (Weissenberg 1955, Stephens et al. 1970); in gobiid and diodontid fishes of the Gulf of Panama and Gulf of California (McCosker and Nigrelli 1971); and in ling cod (Ophiodon elongatus) from the Hecate Strait, British Columbia (Walker 1947).

The gross lesion found on English sole in this study was present as multiple white to pale yellowish ovoid surface nodules 0.5 to 2 mm in diameter, present only on the blind side pectoral fin; the fin most often in contact with bottom sediments. The histopathologic features of the nodules included massively hypertrophied cells of a fibroblastic nature directly beneath the stratified epithelium of the fin epidermis. Each individual hypertrophied cell was enclosed by a distinctive hyaline capsule. These cells had a large nucleus undergoing karyolysis and a granular cytoplasm containing basophilic and Feulgen-positive material comprising the viral inclusion body (Fig. 3). Microscopic appearance of this lesion was consistent with the features of lymphocystis disease reported for other fish species (Wellings et al. 1977, Nigrelli et al. 1965).

A portion of a nodule from the fin of the affected English sole was reprocessed for examination by electron microscopy. The viral particles were well-preserved, and displayed the morphology typical of that previously described for lymphocystis virus (Alpers et al. 1977a, Walker and Weissenberg 1965, Fig. 4).

The diameter of the nucleocapsid ranged from 180 to 210 nm. This corresponds closely to the 180-220 nm nucleocopsid measurements reported for lymphocystis virus particles in yellowfin sole (Alpers et al. 1977a). Also present in the cytoplasm were numerous randomly distributed fibrillar structures (Fig. 5) similar to those described by Alpers et al. (1977a). These fibrillar structures displayed a banding periodicity of approximately 800 Å periodicity also reported by Alpers et al. (1977a). This periodicity does not coincide with the value associated with collagen (640 Å), and the tapered appearance is not typical of the appearance of collagen. These structures were not found in normal fibroblasts, and their presence may be associated with the process of viral infection.

Angioepithelial Nodules (AENs)

The AEN was grossly visible as a hemispherical, smooth-surfaced, red to pale pink nodule, on the skin furface, measuring 1-10 mm in diameter. Multiple tumors were often observed. Microscopic features were increased vascularization of the stratum spongiosum (subepidermal layer) with hyperemia, prominent mononuclear infiltrate, increased connective tissue elements, foci of coagulation necrosis, all indicative of a typical pleuronectid inflammatory response. The overlying epidermis was typically hyperplastic, but not papillomatous or folded. Within these areas of dermal inflammation and congestion were numerous extravascular cells identical in appearance to the so-called X-cell associated with this disease complex (Brooks et al. 1969, Wellings et al. 1976b). In the AEN, the X-cell had a prominent karysosome within a large nucleus, a granular, vacuolated cytoplasm, a cell diameter of approximately 15 μ, and was typically surrounded by reactive macrophages and extravasated red blood cells. Only rarely were X-cells visible in the overlying epidermis. The swelling and redness in this tumorous lesion was due to the increased vascularization, hyperemia, and inflammatory response, and not to multiplication of X-cells.

The transitional form of AEN was characterized by a similar but reduced dermal hyperemia, mononuclear (lymphocytes and macrophages) infiltrate, and connective tissue proliferative response, but in contrast to the AEN the epidermis was not only thickened but was folded (papilliform) and appeared to contain multiple nests of X-cells surrounded by a wavy, folded fibrovasular stroma. The stroma typically contained large melanophores, and often a dense mononuclear cell infiltrate. A thin cap (2-3 cell layer thick) of epidermal cells overlay the multiple clusters of X-cells.

Grossly, the fully developed epidermal papillomas were pink to grey to brown in color depending on the vascularity of the tumor and the density of melanophages in the stroma. The outer cutaneous surfaces were papillomatous in appearance, composed of interdigitating, folding ridges of tissue. These low lying tumors were found anywhere on the external body surface, fins, and opercula, spreading in a sheet-like fashion. They were quite variable in size and shape, but generally were up to 5 cm in diameter, and 3-4 mm in depth. Often the tumors were multiple.

Microscopically, the epidermal papillomas were composed of folded ridges of a highly thickened epidermis-like tissue underlain by the stratum spongiosum of the dermis. Multiple nests of X-cells of variable size, bordered by a basal lamina, and supported by a fibrovascular stroma containing melanophores formed the bulk of the tumor tissue. The thin cap of epidermal malphigian cells overlayed the papilliform folds.

The stroma was typically populated by mild to moderate infiltrate of mononuclear leucocytes, which were also occasionally present within the X-cell nests. When present as focal aggregates, these inflammatory cells were usually associated with degenerate, vacuolated forms of X-cells at a central position within the nest.

REFERENCES

Agius, C. 1979. The role of melano-macrophage centers in iron storage in normal and diseased fish. Journal of Fish Diseases 2:337-343.

Alpers, C.E., B.B. McCain, M.S. Myers and S.R. Wellings. 1977a. Lymphocystis disease in yellow in sole (Limanda aspera) in the Bering Sea. Journal of the Fisheries Research Board of Canada. 34(5):611-616.

Alpers, C.E., B.B. McCain, M.S. Myers, S.R. Wellings, M. Poore, J. Bagshaw and C.J. Dawe. 1977b. Pathologic anatomy of pseudobranch tumors in Pacific cod, *Gadus macrocephalus*. Journal of the National Cancer Institute 59:377-398.

Baird, W.M., C.J. Chern and L. Diamond. 1977. Formation of benzo[a] pyrene glucuronic acid conjugates in hamster embryo cell cultures. Cancer Research 37:3190-3197.

Bannasch, P. 1976. Cytology and cytogenesis of neoplastic (hyperplastic) hepatic nodules. Cancer Research 36:2555-2562.

Bannasch, P. 1978. Sequential cellular alterations during hepatocarcinogenesis. In, *Rat Hepatic Neoplasia*, P.M. Newberne and W.H. Butler, eds. MIT Press, Cambridge. pp. 58-93.

Brookes, P. 1977. Mutagenicity of polycyclic aromatic hydrocarbons. Mutation Research 39:257-284.

Brooks, R.E., G.E. McArn and S.R. Wellings. 1969. Ultrastructural observations on an unidentified cell type found in epidermal tumors of flounders. Journal of the National Cancer Institute 43:97-110.

Brown, D.W., L.S. Ramos, M.Y. Uyeda, A.J. Friedman and W.D. MacLeod, Jr. 1980. Ambient-temperature extraction of hydrocarbons from marine sediment: Comparison with boiling-solvent extractions. Advances in Chemistry Series, American Chemical Society. 14:313-326.

Chitwood, M. and J.R. Lichtenfels. 1972. Identification of parasitic metazoa in tissue sections. Experimental Parasitology 32:407-519.

Dawe, C.J. 1980. Polyoma tumors in mice and X-cell tumors in fish, viewed through telescope and microscope. Eleventh International Symposium of Princess Takamatsu Cancer Research Fund, Nakahara Memorial Lecture.

Dawson, W.A. and L.J. Tilly. 1972. Measurement of salt-wedge excursion distance in the Duwamish River estuary, Seattle, Washington, by means of dissolved oxygen gradient. U.S. Geological Survey Water-Supply Paper, 1873-d. Prepared in cooperation with the Municipality of Metropolitan Seattle. 27 pp.

Edmondson, H.A. 1958. Tumors of the liver and intrahepatic bile ducts. In, Atlas of Tumor Pathology, Armed Forces Institute of Pathology, Section VII - Fasicle 25. 216 pp.

Falkmer, S., S. Marklund, P.E. Mattson and C. Rappe. 1977. Hepatomas and other neoplasms in the Atlantic hagfish, Myxine glutinosa: A histopathologic and chemical study. Annals of the New York Academy of Sciences 298:342-355.

Farber, E. 1956. Similarities in the sequence of early histological changes induced in the liver by ethionine, 2-acetylaminofluorene and 3' methyl-4-dimethyl aminoazo-benzene. Cancer Research 16:142-149.

Farber, E. 1976. On the pathogenesis of experimental hepatocellular carcinoma. In, Hepatocellular Carcinoma, K.O. Kuda and R.L. Peters, eds. John Wiley and Sons, New York. pp. 1-24.

Farber, E., S. Parker and M. Greenstein. 1976. The resistance of putative premalignant liver cell populations, hyperplastic nodules, to the acute cytotoxic effects of some hepatocarcinogens. Cancer Research 36:3879-3887.

Farber, E., D. Solt, R. Cameron, B. Laishes, K. Ogawa and A. Medline. 1977. Newer insights into the pathogenesis of liver cancer. American Journal of Pathology 89:477-482.

Hart, J.L. 1973. Pacific Fishes of Canada. Fisheries Research Board of Canada, Bulletin 180. 740 pp.

Hendricks, J.D., R.O. Sinnhuber, J.E. Dixon, J.H. Wales, and M.S. Masri and D.P.H. Hsieb. 1980. Carcinogenic response of rainbow trout, (Salmo gairdneri). Aflatoxin Q. and synergistic effect of cyclopropenoid fatty acids. Journal of the National Cancer Institute 64(3):523-527.

Heston, W.E., G. Vlahakis and M.K. Deringer. 1960. High incidence of spontaneous hepatomas and the increase of this incidence with urethan in CBH, C3Hf and C3He male mice. Journal of the National Cancer Institute 24:425.

Hinton, D.E., J.E. Klaunig and M.M. Lipsky. 1978. PCB-induced alterations in teleost liver: A model for environmental disease in fish. Marine Fisheries Review 40(10):47-50.

Hoffman, G.L. 1970. Parasites of North American Freshwater Fishes. University of California Press, Berkeley. 486 pp.

Hoffman, G.L., C.E. Dunbar, K. Wolf and L.O. Zwillenberg. 1969. Epitheliocystis, a new infectious disease of the bluegill, Lepomis macrochirus. Antonie von Leeuwenhoek 35:146-158.

Houston, A.H. and M.A. Dewilde. 1969. Environmental temperature and the body fluid system of the freshwater teleost. III. Hematology and blood volume of thermally acclimated brook trout, Salvelinus fontinalis. Comparative Biochemistry and Physiology 28:877-885.

Ito, N., H. Nagasaki, M. Arai, S. Makiura, S. Sugihara and H. Kazuya. 1973a. Histopathologic studies of liver tumorigenesis induced in mice by technical polychlorinated biphenyls and its promoting effect on liver tumors induced by benzene hexachloride. Journal of the National Cancer Institute 51:1637-1646.

Ito, N., H. Nagasaki, M. Arai, S. Sugihara and S. Makiura. 1973b. Histologic and ultrastructural studies of the hepatocarcinogenicity of benzene hexachloride in mice. Journal of the National Cancer Institute 51:817-826.

Ito Y., I. Kimura and T. Miyake. 1976. Histopathological and virological investigations of papillomas in soles and gobies in coastal waters of Japan. Progress in Experimental Tumor Research, Karger, Basel. 20:86-93.

Jago, M.V. 1969. The development of the hepatic megalocytosis of chronic pyrrolizidine alkaloid poisoning. American Journal of Pathology 56(3):405-421.

Jones, G. and W.H. Butler. 1975. Morphology of spontaneous and induced neoplasia. In, Mouse Hepatic Neoplasia, W.H. Butler and P.M. Newberne, eds. Elsevier Scientific Publishing Co., Amsterdam. pp. 21-60.

Jubb, K.V.F. and P.C. Kennedy. 1970. Pathology of Domestic Animals. 2nd Edition, Vol 2. Academic Press, New York. pp. 216-219.

Karasaki, S. 1976. Ultrastructural and cytochemical studies on hyperbasophilic foci with special reference to the demonstration of cells surface alterations in hepatocarcinogenesis. Cancer Research 36:2567-2572.

Kawamoto, S., A. Kirschbaum, M.L. Ibanez, J.J. Trentin and H.G. Taylor. 1961. Influence of urethane on the development of spontaneous leukemia and on the induction of hemangiomas in the AKR and C58 strains of mice. Cancer Research 21(1):71.

Kimbrough, R.D., R.E. Linder and T.B. Gaines. 1972. Morphological changes in livers of rats fed polychlorinated biphenyls. Archives of Environmental Health 25:354-364.

Kimbrough, R.D. and R.E. Linder. 1974. Induction of adenofibrosis and hepatomas of the liver in BALB/CJ mice by polychlorinated biphenyls. Journal of the National Cancer Institute 53:547-552.

Kimbrough, R.D., R.A. Squire, R.E. Linder, J.D. Straudberg, R.J. Montali and V.W. Burse. 1975. Induction of liver tumors in Sherman strain female rats by the polychlorinated biphenyl Arochlor 1260. Journal of the National Cancer Institute 55:1453-1459.

Kimura, N.T. and T. Baba. 1973. Neoplastic changes in the rat liver induced by polychlorinated biphenyls. GANN 64:105-108.

Kitigawa, T. 1976. Sequential phenotypic changes in hyperplastic areas during hepatocarcinogenesis in the rat. Cancer Research 36:2534-2539.

Koller, L.D. and J.G. Zinkl. 1973. Pathology of polychlorinated biphenyls in rabbits. American Journal of Pathology 70(3):363-377.

Krumbein, W.C. and F.J. Pettijohn. 1938. Manual of Sedimentary Petrography. The Twentieth Century Earth Science Series, Kirtley F. Mather, ed. Appleton-Century-Crofts, Inc., New York. 549 pp.

Lakowicz, J.R., D.R. Beran and S.C. Reimer. 1980. Transport of a carcinogen benzo[a]pyrene from particulates to lipid bilayers. A model for the fate of particle-absorbed polynuclear aromatic hydrocarbons which are retained in the lungs. Biochimica Biophysica Acta 629:243-258.

Lange E. and J.V. Johanessen. 1977. Histochemical and ultrastructural studies of chemodectoma-like tumors in the cod, *Gadus morhua* L., Laboratory Investigation 37(1):96-104.

Larsson, A., B.E. Bengsston and O. Svanberg. 1976. Some hematological and biochemical effects of cadmium on fish. In, Effect of Pollutants on Aquatic Organisms, A.P.N. Lockwood, ed. Society for Experimental Biology Seminar Vol. 2. Cambridge University Press, Cambridge. pp. 35-45.

Luna, L.G. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd Edition. McGraw-Hill Book Company, New York. 258 pp.

Malevski Y., J.H. Wales and M.W. Montgomery. 1974. Liver damage in rainbow trout, *Salmo gairdneri*, fed cyclopropenoid fatty acids. Journal of the Fisheries Research Board of Canada 31:1397-1400.

Malins, D.C., T.K. Collier and H.R. Sanborn. 1979. Disposition and metabolism of aromatic hydrocarbons in marine organisms. In, Pesticide and Xenobiotic Metabolism in Aquatic Organisms, M.I.Q. Khan, J.J. Lech and J.J. Menn, eds. American Chemical Society, Washington, D.C. pp. 57-75.

Malins, D.C., B.B. McCain, D.W. Brown, A.K. Sparks and H.O. Hodgins. 1980. Chemical contaminants and biological abnormalities in Central and Southern Puget Sound. NOAA Technical Memorandum OMPA-2. 295 pp.

Margolis, L. and J.R. Arthur. 1979. Synopsis of the parasites of fishes of Canada. Fisheries Research Board of Canada, Bulletin 199. 269 pp.

Martin, B.J. 1980. Effects of petroleum compounds on estuarine fishes. Ecological Research Series EPA-600/3-80-01. pp. 1-31.

McArn, G.E., R.E. Chuinard, B.S. Miller, R.E. Brooks and S.R. Wellings. 1968. Pathology of skin tumors found on English sole and starry flounder from Puget Sound, Washington. Journal of the National Cancer Institute 41:229-242

McCain, B.B., K.V. Pierce, S.R. Wellings and B.S. Miller. 1977. Hepatomas in marine fish from urban estuary. Bulletin of Environmental Contamination and Toxicology 18:1-2.

McCain B.B., M.S. Myers, W.D. Gronlund, S.W. Wellings and C.E. Alpers. 1978a. The frequency, distribution and pathology of three diseases of demersal fishes in the Bering Sea. Journal of Fish Biology 12:267-276.

McCain, B.B., H.O. Hodgins, W.D. Gronlund, J.W. Hawkes, D.W. Brown, M.S. Myers and J.H. Vandermeulen. 1978b. Bioavailability of crude oil from experimentally oiled sediments to English sole (Parophrys vetulus), and pathological consequences. Journal of the Fisheries Research Board of Canada 35(5):657-664

McCain, B.B., W.D. Gronlund, M.S. Myers and S.R. Wellings. 1979. Tumors and microbial diseases of marine fishes in Alaskan waters. Journal of Fish Diseases 2:111-130.

McCain, B.B. and D.C. Malins. 1981. Effects of petroleum hydrocarbons on selected demersal fish and crustaceans. In, Ecological Stress and the New York Bight: Science and Management. Published by New York Bight Marine Ecosystems Analysis (MESA), in press.

McCosker, J.E. and R.F. Nigrelli. 1971. New records of lymphocystis disease in four eastern Pacific fish species. Journal of Fisheries Research Board of Canada 28:1809-1810.

McCosker, J.E., M.D. Lagios and T. Tucker. 1976. Ultrastructure of lymphocystis virus in the Quillback rock fish (Sebastes maliger) with records of infection in other aquarium-held fishes. Transactions of the American Fisheries Society 2:333-337.

McLean, E.K. 1970. The toxic action of pyrrolizidine (*Senecio*) alkaloids. Pharmacology Review *22*:429.

McLean, E.K. 1974. *Senecio* and other plants as liver poisons. In, *International Symposium on Hepatotoxicity*. Academic Press, New York. pp. 142-146.

Morita, T., I. Kihara, T. Oite, T. Yamamoto and Y. Suzuki. 1978. Mesangiolysis: sequential ultrastructural study of Habu venom-induced glomerular lesions. Laboratory Investigation *38*(1):94-102.

Moulton, J.E. 1978. *Tumors in Domestic Animals*. University of of California, Berkeley. 2nd Edition. pp. 279-281.

Myers, M.S. 1981. Pathologic anatomy of papilloma-like tumors in Pacific Ocean perch (Sebastes alutus) from the Gulf of Alaska. M.S. Thesis, University of Washington. 98 pp.

Newberne, P.M. 1976. Experimental hepatocellular carcinogenesis. Cancer Research *36*:2573-2578.

Newberne, P.M. and W.H. Butler. 1978. *Rat Hepatic Neoplasia*. MIT Press, Cambridge. 288 pp.

Nigrelli, R.F., K.S. Ketchen and S.J. Ruggieri. 1965. Studies on virus diseases of fish. Epizootiology of epithelial tumors in skin of flatfishes of the Pacific Coast; with special reference to the sand sole from northern Hecate strait, B.C., Canada. Zoologica (New York) *50*(3):115-122.

Nishizumi, M. 1970. Light and electron microscope study of chlorobiphenyl poisoning in mouse and monkey liver. Archives of Environmental Health *21*:620-632.

Olson, R.E. 1978. Parasitology of the English sole, *Parophrys vetulus* Girard in Oregon, U.S.A. Journal of Fish Biology *13*:237-238.

Ostle, B. 1972. *Statistics in Research*. Iowa State University Press, Ames. 585 pp.

Paperna, I. 1977. Epitheliocystis infection in wild and cultured seabream (*Sparus aurata*, Sparidae) and grey mullets (*Liza ramada*, Mugilidae). Aquaculture *10*:169-176.

Paperna, I., I. Sabnai and M. Castel. 1978. Ultrastructural study of epitheliocystis organisms from gill epithelium of the fish *Sparus auraota* and *Liza ramada* (Risso) and their relation to the host cell. Journal of Fish Diseases *1*:181-189.

Pattie, B.H. 1975. Estuarine monitoring program (July 1, 1974 - June 30, 1975). Project Completion Report, State of Washington Department of Fisheries, Marine Program. 37 pp.

Pavlou, S.P., K.A. Korgslund, R.N. Dexter and J.R. Clayton. 1973. Data report for the National Coastal Pollution Research Program, University of Washington, Seattle. Special Report. No. 54. 178 pp.

Pavlou, S.P. and W. Hom. 1979. PCB removal from the Duwamish River estuary: Implications to the management alternative for the Hudson River PCB cleanup. In, Health Effects of Halogenated Hydrocarbons, W. Nicholson and J. Moore, eds., Annals of the New York Academy of Sciences, N.Y. 320:69-124.

Pierce, K.V., B.B. McCain and S.R. Wellings. 1978. The pathology of hepatomas and other liver abnormalities in English sole (Parophrys vetulus) from the Duwamish River, Seattle, Washington. Journal of the National Cancer Institute 60:1445-1453.

Pitot, H.C. 1977. The natural history of neoplasia. American Journal of Pathology 89(2):402-411.

Preece, A. 1972. A Manual for Histologic Technicians. 3rd Edition. Little, Brown and Co., Boston. 428 pp.

Rabes, H.M., P. Scholze and B. Jantsch. 1972. Growth kinetics of diethylnitrosamine-induced enzyme deficient "preneoplastic" liver cell populations in vivo and in vitro. Cancer Research 32:2577-2586.

Ramos, L.S. and P.G. Prohaska. 1981. Sephadex LH-20 chromatography of extracts of marine sediment and biological samples for the isolation of polynuclear aromatic hydrocarbons. Journal of Chromatography 211:284-289.

Reuber, M.D. 1965. Development of preneoplastic and neoplastic lesions of the liver in male rats given 0.025 percent N-2 fluorenydiacetamide. Journal of the National Cancer Institute 34:697-724.

Reuber, M.D. 1968. Histogenesis of cholangiofibrosis and well-differentiated cholangiocarcinoma in Syrian hamsters given Z-acetamidofluorene or 2-diacetamidoflourene. GANN 59:239-246.

Robbins, S.L. and R.S. Cotran. 1979. Pathologic Basis of Disease. 2nd Edition. W.B. Saunders Company, Philadelphia. 1598 pp.

Roberts, R.J. 1975. Melanin containing cells of teleost fish and their relation to disease. In, The Pathology of Fishes, W.E. Ribelin and G. Migaki, eds. University of Wisconsin Press, Madison. pp. 477-496.

Roe, F.J.C. 1954. Liver changes in urethane-treated mice appearing after a long latent interval. 32nd Annual Report of the British Empire Cancer Campaign. 170 pp.

Salt, G. 1970. The cellular defense reactions of insects. Cambridge monographs in experimental biology, #16. Cambridge University Press. 118 pp.

Scherer, E. and P. Emmelot. 1975. Kinetics of induction and growth of precancerous liver cell foci and liver tumor formation by diethylnitrosamine in the rat. European Journal of Cancer 11:689-696.

Scherer, E. and P. Emmelot. 1976. Kinetics of induction and growth of enzyme-deficient islands involved in hepatocarcinogenesis. Cancer Research 36:2544-2554.

Schillings. P.H.M. and J.H.S. Stekhoven. 1980. Atlas of Glomerular Histopathology. S. Karger, (Basel, New York). 143 pp.

Sherwood, M.J. and B.B. McCain. 1976. Comparison of fin erosion disease: Los Angeles and Seattle. Annual Report, Southern California Coastal Water Research Project, El Segundo, California.

Sims, P., P.L. Grover, A. Swaisland, K. Pal and A. Hewer. 1974. Metabolic activation of benzo[a]pyrene proceeds by a diol-epoxide. Nature (London) 252:326-328.

Sinnhuber, R.O., D.J. Lee, H.H. Wales and J.L. Ayres. 1968. Dietary factors and hepatoma in rainbow trout (Salmo gairdneri). II. Cocarcinogenesis by cyclopropenoid fatty acids and the effect of gossypol and altered lipids on aflatoxin-induced liver cancer. Journal of the National Cancer Institute 41:1293-1301.

Sinnhuber, R.O., J.D. Hendricks, J.H. Wales and G.B. Putman. 1977. Neoplasms in rainbow trout, a sensitive animal model for environmental carcinogenesis. In, Aquatic Pollutants and Biologic Effects with Emphasis on Neoplasia, H.F. Kraybill, C.J. Dawe, J.C. Harshbarger, R.G. Tardiff, eds. Annals of the New York Academy Sciences 298:389-408.

Smith, C., T.H. Peck, R.J. Klauda and J.B. McLaren. 1979. Hepatomas in Atlantic tomcod Microgadus tomcod (Walbaum) collected in the Hudson River estuary in New York. Journal of Fish Diseases 2:313-319.

Sokal, R. and F. Rohlf. 1969. Biometry. W.H. Freeman and Co., San Francisco. 776 pp.

Solt, D.B., A. Medline and E. Farber. 1977. Rapid emergence of carcinogen-induced hyperplastic lesions in a new model for the sequential analysis of liver carcinogenesis. American Journal of Pathology 88:595-618.

Squire, R.A. and M.H. Levitt. 1975. Report of a workshop on classification of specific hepatocellular lesions in rats. Cancer Research 35:3214-3223.

Stephens, J.S., Jr., R.K. Johnson, G.S. Key, and J.E. McCosker. 1970. The comparative ecology of three sympatric species of California blennies of the genus Hypsoblennius (Teleostomi Blenniidae). Ecological Monographs 40(2)213-233.

Struthers, B.J., J.H. Wales, D.J. Lee and R.O. Sinnhuber. 1975. Liver composition and histology of rainbow trout fed cyclopropenoid fatty acids. Experimental and Molecular Pathology 23:164-170.

Thompson, S.W. 1966. Selected Histochemical and Histopathological Methods. Charles C. Thomas, Springfield, Illinois. 1639 pp.

Varanasi, U., M. Uhler and S.I. Stranahan. 1978. Uptake and release of napthalene and its metabolites in skin and epidermal mucus of salmonids. Toxicology and Applied Pharmacology 44:277-289.

Varanasi, U., D.J. Gmur and P.A. Treseler. 1979. Influence of time and mode of exposure on biotransformation of naphthalene by juvenile starry flounder (Platichthys stellatus) and rock sole (Lepidopsetta bilineata). Archives of Environmental Contamination and Toxicology 8:673-692.

Varanasi, U. and D.J. Gmur. 1981a. Hydrocarbons and metabolites in English sole (Parophrys vetulus) exposed simultaneously to [H^3] benzo[a] pyrene and [^{14}C] naphthalene in oil-contaminated sediment. Aquatic Toxicology 1:49-67.

Varanasi, U. and D.J. Gmur. 1981b. In vivo metabolism of naphtalene and benzo[a]pyrene by flatfish. In, Chemical Analysis and Biological Fate: Polynuclear Aromatic Hydrocarbons, M. Cook and A.J. Dennis, eds. Fifth International Symposium on Polynuclear Aromatic Hydrocarbons, Battelle Memorial Institute. Battelle Press, Columbus. pp. 367-376.

Walker, R. 1947. Lymphocyctis disease and neoplasia in fish. Report of lymphocystis in Ophiodon elongatus from the Straits of Georgia, B.C. Anatomical Record 99:559-560 .

Walker, R. and R. Weissenberg. !965. Conformity of light and electron microscopic studies on virus particle distribution in lymphocystis tumor cells of fish. Archives of the New York Academy of Sciences 126:375-385.

Ward, J.M. and G. Vlahakis. 1978. Evaluation of hepatocellular neoplasms in mice. Journal of the National Cancer Institute 61:807-811.

Wehner, H. 1968. Stereologische untersuchungen am mesangium normaler menschlicher nieren. Virchows Arch. Abt. A. Path Anat. 344:286-194.

Weisburger, J.H., R.S. Yamamoto, G.M. Williams, P.H. Grantham, T. Matsushima and E.K. Weisburger. 1972. On the sulfate ester of N-hydroxy-N-2-fluyorenylactamide as a key ultimate hepatocarcinogen in the rat. Cancer Research 32:491-500.

Weissenberg, R. 1955. The third spontaneous case of lymphocystis virus disease of fish from the Pacific Coast of North America. Anatomical Record (Abstract) 122(3):434-435

Wellings, S.R., B.B. McCain, and B.S. Butler. 1976a. Epidermal papillomas in Pleuronectidae of Puget Sound, Washington. Progress in Experimental Tumor Research 20:55-74.

Wellings, S.R., C.E. Alpers, B.B. McCain, and B.S. Miller. 1976b. Fin erosion disease of starry flounder (*Platichthys stellatus*) and English sole (*Parophrys vetulus*) in the estuary of the Duwamish River, Seattle, Washington. Journal of the Fisheries Research Board of Canada 33:2577-2586

Wellings, S.R., C.E. Alpers, B.B. McCain and M.S. Myers. 1977. Fish disease in the Bering Sea. In, *Aquatic Pollutants and Biologic Effects with Emphasis on Neoplasia*, H.F. Kraybill, C.J. Dawe, J.C. Harshbarger, R.G. Tardiff, eds. Annals of the New York Academy of Sciences 298:290-304.

Williams, G.M. 1976. Functional markers and growth behavior of preneoplastic hepatocytes. Cancer Research 36:2540-2543.

Wingert, R.C., B.B. McCain, K.V. Pierce, S.F. Borton, D.T. Griggs and B.S. Miller. 1976. Ecological and disease studies of demersal fishes in the vicinity of sewage outfalls. 1976 Research in Fisheries. Annual Report of the College of Fisheries, University of Washington, Seattle.

Word, Jack Q. 1976. Biological comparison of grab sampling devices. Annual Report, Southern California Coastal Water Research Project, Long Beach, California. pp. 189-194

Zachary, A. and I. Paperna. 1977. Epitheliocystis disease in the striped bass *Morone saxatilis* from the Chesapeake Bay. Canadian Journal of Microbiology 23:1404-1414.

Zar, J.H. 1974. *Biostatistical Analysis*. Prentice-Hall, Inglewood Cliffs New Jersey. 620 pp.

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